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Biochimica et Biophysica Acta 1719 (2005) 36 – 58
BIOCHIMICA ET BIOPHYSICA ACTA
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Review

Pharmacology of Gap junctions. New pharmacological targets for treatment of arrhythmia, seizure and cancer?

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Received 14 June 2005; received in revised form 25 August 2005; accepted 6 September 2005
Available online 21 September 2005

Abstract

Intercellular communication in many organs is maintained via intercellular gap junction channels composed of connexins, a large protein family with a number of isoforms. This gap junction intercellular communication (GJIC) allows the propagation of action potentials (e.g., in brain, heart), and the transfer of small molecules which may regulate cell growth, differentiation and function. The latter has been shown to be involved in cancer growth: reduced GJIC often is associated with increased tumor growth or with de-differentiation processes. Disturbances of GJIC in the heart can cause arrhythmia, while in brain electrical activity during seizures seems to be propagated via gap junction channels. Many diseases or pathophysiological conditions seem to be associated with alterations of gap junction protein expression. Thus, depending on the target disease opening or closure of gap junctions may be of interest, or alteration of connexin expression. GJIC can be affected acutely by changing gap junction conductance or – more chronic – by altering connexin expression and membrane localisation. This review gives an overview on drugs affecting GJIC.

Keywords: Gap junction; Connexin; Intercellular communication; Pharmacology; Toxicology; Cardiac; Cancer; Seizure

Contents

1. Introduction

Pharmacology is the science of the interaction of substances, usually called drugs, with living organisms. The clinical aspect of this science is the use of a certain interaction to influence the pathophysiology of a disease. A drug is any chemical agent affecting processes of living. This broad definition includes an extensive number of agents and mechanisms of action. A certain aspect is that – to speak with Paracelsus – "every agent is a poison, nothing is without poison and it is solely the dose which makes that an agent is not a poison". That means – and this will be important for the issue of pharmacology of gap junctions – that a drug may have beneficial effects at low doses and may be deleterious at higher doses. This might be of

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doi:10.1016/j.bbamem.2005.09.007

serious importance when the intercellular communication is affected.

While the classical pharmacological approach was starting at the traditional evidence from older times that a certain medicine has been used for treatment of a disease with subsequent purification of the active agent and characterization of the mechanism of action, modern pharmacology starts with the definition of a drug target, i.e., a structure, mostly a protein, which is believed to play a key role in the pathophysiology of a disease. Subsequently, an innumerate amount of agents is tested by screening assays for its efficacy at the target structure (often using binding assays), followed by a test of the effective agents in certain cell culture or organ models, to define whether they have agonist or antagonistic affects, how they affect the physiology of the organ and whether they might influence the pathomechanism of the disease.

In the last years it has become clear that cells forming an organ communicate with each other and that this intercellular communication is a basic prerequisite for proper organ function. Previously, communication was known via nerves, via hormones and mediators. Here we are facing another type of communication, the direct cell-to-cell communication via gap junctions (gap junction intercellular communication; GJIC), realized via intercellular gap junction channels, formed by two hemichannels provided by either of the two neighboring cells. Each hemichannel, or connexon, is a hexameric pore structure made from 6 protein subunits, the connexins. At present, the connexins (Cx) comprise a gene family of 20 members in mouse and 21 in human [1] (hCx23, hCx25, hCx26, hCx30.2, hCx30, hCx31.9, hCx30.3, hCx31, hCx31.1, hCx32, hCx36, hCx37, hCx40.1, hCx40, hCx43, hCx45, hCx46, hCx47, hCx50, hCx59, hCx62; h=human, Cx=connexin, the number gives the approximate molecular weight in kDa). The various connexin isoforms differ with regard to their molecular weight due to different lengths of their C-terminals. A connexin is a protein with 4 transmembrane spanning domains, two extracellular and 1 intracellular loop, and with N- and C-terminal at the intracellular side [2]. Gap junction channels allow the propagation of an electrical impulse (action potential) and the transfer of small molecules (up to 1000 Dalton). Connexins are synthetised in the rough endoplasmic reticulum, folded and inserted into vesicles for transfer to the Golgi apparatus, where they are oligomerized to hexameric hemichannels. Thereafter, they are transported along micotubular structures to the plasma membrane and dock to another hemichannel of the neighboring cell thus forming a complete gap junction channel (see [3–5]. This process includes interaction with the cytoskeletal apparatus, in particular with zonula occludens protein 1 ZO-1 (for review, see [6]). Connexins are degraded with short half-life times (see above) via either the lysosomal or (predominantly) the proteasomal pathway.

Intercellular coupling can be regulated by either the number of channels as well as by the mean open time, the mean closed time, and the single channel conductance. The number of channels is either controlled by the rate of synthesis of connexins, their transport from the sarcoplasmic reticulum to the *trans*-Golgi network [3] and their integration and assembly in the membrane [7], or by their degradation [8]. The half life time of these channels is with 1–5 h rather short [9,10] (Cx43: about 90 min; Cx45 about 2.9 h: [11]). Function and life-time of connexins including connexin trafficking, assembly, disassembly, degradation and control of gating are controlled – at least partially – by phosphorylation [12].

2. General approaches

Pharmacology of GJIC is still at its beginnings and we need to define agents affecting GJIC for a subsequent test of these agents in various models of disease. In the following paragraphs we summarize the classes of agents and the types of action which have been shown to affect GJIC. We will first describe mechanisms and drugs acting on gap junction conductance rather than on number of channels, section 1 describing those drugs which reduce coupling, section 2 those enhancing coupling. This will be followed by a third section on drugs and mechanisms known to act on the number of channels by acting on expression, synthesis, trafficking, docking or degradation. Finally we will focus on possible new perspectives for the treatment of diseases (Fig. 1).

Before starting with these a small excurse on ionic mechanisms should be given which is necessary for the understanding:

2.1. Ions

There is no certain class of drugs affecting gap junctions. In contrast, many agents can affect the channels: Thus, increasing concentrations of ions such as Na^+, Ca^{++}, Mg^{++}, and H^+ can reduce acutely gap junction conductance within minutes [13–15]. Regarding Ca^{++}, reduction in g_j occurs if the intracellular calcium concentration exceeds the range 320–560 nmol/l [16]. Intracellular acidification is known to decrease junctional electrical coupling in cardiomyocytes and in Purkinje fibres [14,17,18]. Regarding the pH sensor, the carboxy tail length has been demonstrated as a determinant of the pH sensitivity [19]. Further investigations [20] revealed a new model of intramolecular interactions in which the carboxy terminal serves as an independent domain which can bind to another separate domain of the connexin protein (e.g., a region including His-95) closing the channel, comparable to the ball-and-chain-model for potassium channels. Cx40 and Cx43 seem to act synergistically and enhance pH sensitivity when coexpressed [21]. Na^+-withdrawal in adult rat cardiomyocytes induced electrical uncoupling within 3 min which has been considered a consequence of impaired Na^+/Ca^{++}-exchange [16]. On the other hand, increases in $[Na^+]_i$ can cause uncoupling within 500 ms in Purkinje fibers [22] which – on the other hand – might be secondary to a rise in intracellular Ca^{++} via the Na^+/Ca^{++}-exchange mechanism. Besides this, Ca^{++} is known to interact with calmodulin. Calmodulin, however, can interact with connexins and plays a role in chemical channel gating [23,24]. According to these studies, voltage-sensitivity and CO_2-asensitivity of Cx45 as well as

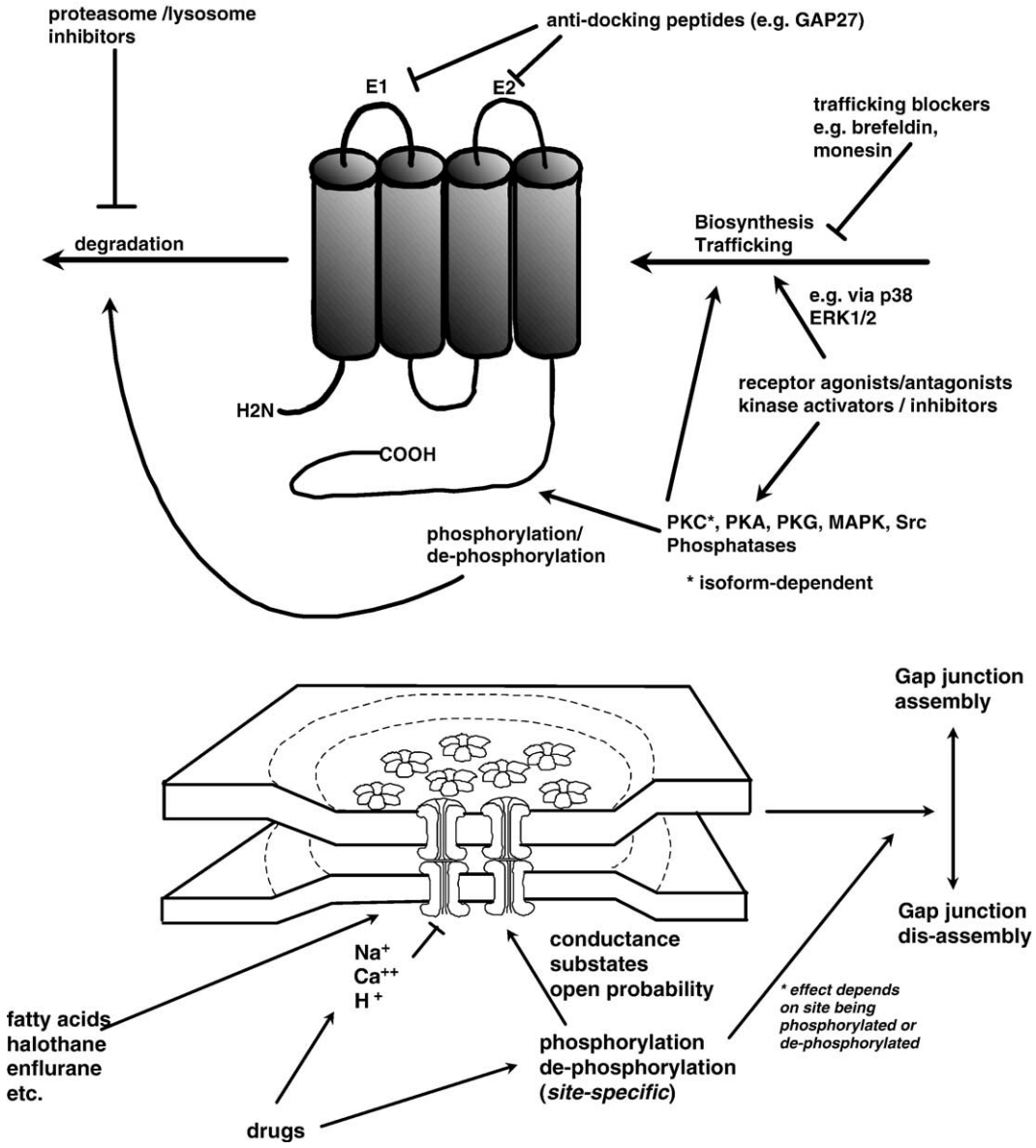

Fig. 1. Schematic view of a gap junction channel and its constituent component, the connexin, as a target of pharmacology.

Cx32 channels is lessened if calmodulin is antagonized. This issue and possible interaction between H+ and Ca++ ions are extensively discussed and reviewed recently by Peracchia [25]. Elevation in $[Mg^{++}]_i$ from 1 to 10 mmol/l at pH=7.4 in the absence of calcium has also been reported to reduce junctional conductance in pairs of adult guinea pig cardiomyocytes [14]. A Hill coefficient of 3.0 was calculated. Since the slope of the pCa-g_j and the pMg-g_j relationships were similar, it was suggested that both divalent cations bind to the same receptor site.

2.2. Drugs used for acute closure of gap junctions (Table 1)

Drugs may affect intracellular ionic homeostasis thereby indirectly altering gap junction conductance. Thus, cardiac glycosides bind to and inhibit the membrane Na/K-ATPase leading to alteration of Na^+/Ca^{++} exchange finally enhancing intracellular concentrations of Na^+ and Ca^{++} which in consequence can uncouple the cells [26]. This uncoupling occurs at nearly therapeutic concentrations of, e.g., 0.68 μM ouabain [22] and, thus, may contribute to the well-known arrhythmogeneity of digitalis. Following this acute uncoupling, later ouabain (in extremely high concentrations of 1 mM) leads to decreased trafficking of connexins with reductions in Cx40 and Cx43 expression [27]. However, it should be noted that the latter action is only of academic interest, since ouabain concentrations of 1 mM are far out of the therapeutic concentration rang (which is in the order of 0.1–0.5 μM) (Table 1).

Regarding the effects of amines, such as histamine, adrenaline and noradrenaline, there are mainly investigations on the coupling-increasing effects of stimulation of cAMP-pathway which typically is activated via β-adrenoceptors [28]; for review, see [29]; and see next section on coupling agents). The role of α-adrenoceptors in regulation of cardiac or vascular gap junction conductance remains unclear. However, in acinar

Table 1
Agents acutely uncoupling cardiovascular gap junctions

Class	Agents	Proposed mechanism	Reported Cx	Ref.
Ions	Na^+, Ca^{++}, Mg^{++}	direct effects?	43	[13–15]
	Ca^{++}	Via calmodulin?	45, 32	[23,24]
	H^+	Ball and chain	40, 43, 32	[17–21]
Drugs/mediators	Cardiac glycosides, ouabain	Elevation of $[Ca^{++}]_i$	43	[22,26]
	α-adrenoceptor stimul. (phenylephrine)	PKC	32?, 43	[30,31]
	Histamine	H_1-receptor/PKC	43	[32]
	IGF-1	PKCγ, disassembly of g_j	43	[44]
	PDGF	PKC	43	[53]
	Angiotensin-II	AT_1-receptor/PKC?	43	[63–66]
	VEGF	Type2-VEGF-receptor Tyrosine kinase /ERK	43	[67,68]
	Thrombin, endothelin, AlF_4^-	Src-tyrosine kinase	43	[69]
	Lysophosphatidic acid	Src-tyrosine kinase	43	[69]
	Carbachol	Ser368-phosphorylation, cGMP/PKG	43	[48,71]
	Acetylcholine	Nitric oxide	32, 26	[73]
	Cholecystokinin-octapeptide	?	32, 26	[74]
	IL-1β	?	32, 43	[75,76]
	Polyamines: spermine, spermidine	?	40	[79]
	Nitric oxide	?	37	[80]
	Quinine, mefloquine	?	36, 45, 50	[84,85]
	Ilmaquinone	?	43 (26, 31, 32)[a]	[51,87]
	Dicoumarol	Reduced phosphorylation	43	[89]
	Fenamates: meclofenamic acid, niflumic acid, flufenamic acid	?	43	[91–93]
	FGF-2	PKCε	43	[42]
Dephosphorylating agents	2,3 butandione monoxime	Unknown mechanism	43	[36]
Phorbol esters	12-O-tetradecanoyphorbol-13-acetate (TPA)	Activation of PKC[b]/Ca^{++}?	26, 31, 32, 36, 43	[50,51,59]
PKC inhibitors	Staurosporine	Inhibition of PKC[b]	43	[62]
Lipophilic agents and fatty acids	Heptanol, octanol	Incorporation?	43	[43,105–107,113–115]
	Arachidonic acid, oleic acid, palmitoleic acid, decaenoic Acid, myristoleic acid	PKC (oleic acid) Reduced open Probability (heptanol)	43	[108,109]
Glycyrrhizic acid metabolites	18-α-glycyrrhetinic acid	Phosphorylation?		
	18-β-glycyrrhetinic acid Carbenoxolone	Aggregation of Cx-subunits?		
Narcotics	Halothane, ethrane, isoflurane	Incorporation? Reduced meanopen time	43, 40	[103]
Metabolites	ATP-decrease	Dephosphorylation	43	[36,39,49]
	Diacylglycerol/ 1-oleoyl-2-acetyl-*sn*-glycerol	Unknown mechanism/disturbance of the lipid bilayer?	43	
Eicosanoids	11,12-epoxyeicosatrienoic acid	ERK1/2	(37, 40, 43)?	[116]
	(late effect) thromboxane A_2	Cx43 internalization	43	[117]
IP_3-receptor blocker	2-aminoethoxydiphenyl borate	Unknown mechanism	43?	[94]
Cannabinoids	Δ^9-tetrahydrocannabinol	ERK1/2	43	[118]

"(37, 40, 43)?" means that experiments have been carried out in endothelial cells which are known to express Cx37, Cx40 and Cx43, but that it has not been clarified which of these Cx is involved.

[a] Only moderate response [51].

[b] Probably depending on the isoform of PKC.

submandibular gland cells of the rat the administration of 10^{-4} mol/l adrenaline elicits a reduction in dye coupling [30], which could not be mimicked with isoprenaline, but could be antagonized by phenoxybenzamine. Thus, the uncoupling effect of adrenaline in this tissue is mediated by stimulation of the α-adrenoceptor. In several tissues, α-adrenergic stimulation leads to uncoupling. Similarly, α-adrenergic stimulation with phenylephrine in adult rat ventricular cardiomyocytes acutely decreases gap junction coupling in a PKC-dependent manner [31]. Histamine, acting on H1-receptors, which also are $G_{q/11}$ coupled, leads to uncoupling and reduced membranous Cx43 obviously involving PKC [32] (H2 effect, see next section) (Table 2).

This leads to the fact that gap junction conductance can be regulated by phosphorylation of the C-term which comprises consensus sites for several kinases such as cAMP-activated PK (PKA), protein kinase C (PKC; cave: various isoforms), PKG, $p34^{cdc2}$, casein kinase 1, mitogen-activated protein (MAP)

Table 2
Agents reported to acutely enhance gap junctional intercellular communication

Class	Agents	Proposed mechanism	Reported Cx	Ref.
cAMP-enhancing drugs	cAMP, forskolin, isoprenaline	PKA	40, 45	[55,119–122]
Antiarrhythmic drugs	Tedisamil	PKA	?	[98]
Antiarrhythmic peptides	AAPnat, AAP10, cAAP10RG,	PKCα	43	[41,128,131–136,132–139]
	HPP-5, ZP123	PKC?	43	
Eicosanoids	11,12-epoxyeicosatrienoic acid (early effect)	PKA	(37, 40, 43)?	[116]
Phorbol ester	TPA	PKC[a]	43	[47,48]
Unsaturated fatty acids	Eicosapentaenoic acid	inhibition of hypoxia-induced tyrosine-kinase-activation	(37, 40, 43)?	[140,141]
Receptor ligands	5-hydroxytryptamine	?	40, 43	[144]
	Histamine	H_2-receptor, PKA	43	[32]

TPA=12-O-tetradecanoylphorbol 13-acetate; HPP-5=N-3-(4-hydroxyphenyl)-propionyl-Pro-Hyp-Gly-Ala-Gly. "(37, 40, 43)?" means that experiments have been carried out in endothelial cells which are known to express Cx37, Cx40 and Cx43, but that it has not been clarified which of these Cx is involved.
[a] Probably depending on the isoform of PKC.

kinase and a protein tyrosine kinase encoded by the viral oncogene v-src ($pp60^{Src}$) have been shown to target connexins (for review, see [33,34]). Phosphatases or conditions like ischemia can lead to de-phosphorylation of connexins (for review, see [35]). Thus, the dephosphorylating agent 2,3 butandione monoxime (BDM) reduces GJIC [36]. However, this was not correlated with a change in the ratio between non-phosphorylated and phosphorylated Cx43, so that the authors concluded, that the BDM effect might be mediated via regulatory proteins associated with Cx43. Dephosphorylation by endogenous protein phosphatases leads to a run down in channel conductance which can be antagonized by phosphatase inhibitors such as okadaic acid [37]. Dephosphorylation of Cx43 in heart has been suggested to be mediated via PP1-like phosphatases [38]. Similarly, loss of $[ATP]_i$ has been reported to result in gap junction uncoupling, so that the spontaneous rundown of gap junction current in double cell patch clamp experiments can be counteracted by addition of ATP to the pipette solution [39]. This mechanism may be involved in cellular uncoupling in the course of cardiac ischemia and thus might contribute to arrhythmogenesis in cardiac ischemia [36]. Ischemia leads to de-phosphorylation of Cx43 which can be blocked by phosphatase inhibitors such as ocadaic acid (all phosphatases) or caliculin (inhibits PP1 and PP4) [38]. Interestingly, ischemic preconditioning leads to PKC-activation, enhanced Cx43 phosphorylation and reduced myocardial damage [40].

Different isoforms of a protein kinase may lead to different responses as has been shown for PKC: while PKCα can phosphorylate Cx43 and enhance GJIC [41], PKCε reduces GJIC (acute effect of FGF-2 exposition) [42]. PKC activation, e.g., by oleic acid was shown to result in gap junction disassembly and Cx43 Ser368 phosphorylation. While this action could be blocked by classical PKC inhibitors, it was not sensitive to specific PKCα-inhibition by Gö6976 [43]. In rabbit lens cells, IGF-1 induced PKCγ-activation leads to Cx43 phosphorylation and decrease in Cx43 and gap junction plaques [44]. PKCγ translocates to caveolin-1 lipid rafts to which Cx43 is associated [45]. In human lens cells, Cx43 also is phosphorylated by PKCγ causing disassembly and loss of Cx43 from the membrane, while PKCα increased Cx43 in the membrane [46]. Much has been written about the effects of PKC or PKA activation (for recent review, see [29]); however, the results are still conflicting: PKC can be directly activated with phorbol esters such as 12-O-tetradecanoyphorbol-13-acetate (TPA) in comparison to the inactive phorbol ester 4α-phorbol-12,13-didecanoate (αPDD). Activation of PKC by these agents was described in some studies to enhance macroscopic gap junction conductance [47,48], while in others there was no effect [49]. In cardiac myocytes, increase as well as decrease in g_j has been observed in pairs of neonatal cardiomyocytes after PKC-activation by TPA [47,50]. In a systematic study, the effect of TPA on various connexins has been evaluated. According to this study TPA (2 h) reduces GJIC in transfected HeLa-cells coupled by Cx26, Cx31, Cx32, Cx36 and Cx43, but not in cells coupled by Cx45 or Cx56 [51]. In liver cells, TPA-dependent PKC activation also involving ERK1/2 leads to reduced GJIC and enhanced Cx43 degradation [52]. Burt and Steele [53] showed that cells coupled via Cx40 were not uncoupled by PDGF in contrast to Cx43-coupled cells, which could be uncoupled by PDGF acting via PKC-dependent Ser368 phosphorylation. Thus, the phorbolester effect depends on the connexin involved and on the other hand on the PKC isoform.

In cardiac tissue several isoforms of PKC are expressed including PKCα, PKCβ, PKCε, PKCξ and PKCγ (rabbit heart; [54]). TPA treatment is assumed to result in a rapid translocation of PKCα and PKCε in cultured neonatal rat cardiac myocytes [55]. Thus, one may argue that not all isoforms contribute to the gap junction regulation and differences between various preparations or tissues may depend on the subtypes of PKC involved. In addition, Kwak et al. [56] found that TPA increases electrical conductance but decreases permeability as assessed by dye coupling in neonatal rat cardiomyocyte gap junction channels. TPA activates both PKCα and PKCε isoforms (which seem to exert opposite effects on g_j) which might explain the diverging results by a different PKAα/PKCε activation ratio. Both PKCα and PKCε have been shown to phosphorylate Cx43, however, only PKCε phosphorylates directly [57] (Table 3).

Table 3
Agents affecting expression, synthesis, assembly, docking and degradation of gap junctions

Class	Agents	Proposed mechanism	Reported Cx	Ref.
Carotenoids	All-*trans* retinoic acid	Posttranslational regulation?	43	[150]
	Lycopene	Enhanced Cx-expression	43	[151]
	TAC-101	Agonism at retinoic acid receptors	43	[152]
	Indolo[3,2-b]carbazole	Reduced Cx-expression	32	[155]
Hormones	Estradiol (17β-estradiol)	Enhanced Cx-expression (normal endometrium)	26	[172]
		ERα, reduced Cx-expression (cancer cells)	26, 32	[157]
		Reduced SE368-phosphoryl.	43	[173]
		Enhanced (vascular) expression	43	[174]
	FSH	Enhanced Cx-expression	43	[175]
	LH	PKA, MAPK, reduced Cx-expression	43	[175]
	Thyroid hormone (T3)	Enhanced Cx-expression	43	[176,177]
Miscellaneous	Kaempferol	Enhanced phosphorylation and expression	43	[146]
	β-sitosterol	Enhanced expression	43	[147]
	Anisomycin	p38-MAPK-activation/reduced Cx32 expression	32	[161]
	Cholesterol	Reduced endothelial Cx-expression	37, 40	[179]
		Enhanced neointimal Cx-expression	43	[178]
	LPS	Reduced Cx-expression	43	[200]
		Enhanced Cx-expression	43	[201]
	Balifomycin A	Enhanced Cx-presence by inhibition of lysosomes	43	[222]
	ALLN, lactacystin, clastolactacystin, and epoxomicin	Enhanced Cx-presence by inhibition of proteasomes	43	[222]
	Ethanol	Reduced Cx-synthesis	43	[196]
	Brefeldin A	Inhibits Cx-transport in the Golgi apparatus	43	[4]
	Monensin	Trapping of Cx in the *trans*-Golgi network	43	[212]
Cancerogens	Wy-14,643, methapyrilene, hexachlorobenzene, 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), chloroform, p-dichlorobenzene	Reduced Cx-expression	32	[158]
Drugs, mediators	Nicotine	nACh-receptor-agonist, reduced Cx-expression	43	[181]
	HMG-CoA-reductase inhibitors (statins)	Enhanced Cx-expression (endothelial cells)	43	[181]
		Reduced Cx-expression (atheroma vascular cells)	43	[180]
		Enhanced Cx-expression (endothelial cells)	37	[179]
	Bicuculline methiodide	GABA(A)-receptor antagonist Enhanced Cx-expression	32, 43	[164]
	Chloroquine, primaquine	Enhanced Cx-presence by inhibition of lysosomes	43	[222]
	TNFα	Reduced Cx-expression	37, 40	[208]
		Enhanced Cx-expression, via p38MAPK (cardiac cells)	43	[203,204,207]
		Reduced promoter activity (HeLa cells)	43	[200]
	Endothelin	ET_A-receptor, ERK1/2, enhanced Cx expression	43	[194]
	Angiotensin	AT_1-receptor, ERK1/2, p38 Enhanced Cx-expression	43	[194]
	VEGF	Enhanced Cx-expression via TGF-β	43	[68]
	bFGF	Enhanced Cx-expression	43	[198]
	Epidermal growth factor EGF	MEK/reduced Cx-expression	43	[166]
		Cx-internalization	43	[167,168]
Second messenger	cAMP, 8-Br-cAMP	PKA; induction of expression	43, 45	[190]
AC activator	Forskolin	cAMP/PKA, induction of expression	43	[191–193]
Extracellular loop peptides	GAP27, GAP26	Inhibition of docking	37, 43	[216,217]
	E1 and E2 peptides	Inhibition of docking	32, 43	[214,215]
	P180–195/Cx43	Inhibition of docking	43	[219]
	P177–192/Cx40	Inhibition of docking	40	[219]
Antisense Oligonucleotides	GTCACCCATGTCTGGGCA	Inhibition of Cx expression	43	[189]
	GTCACCCATCTTGCCAAG	Inhibition of Cx expression	40	[189]

AC=adenylylcyclase; ALLN=acetyl-leucyl-leucyl-norleucinal; bFGF=basic fibroblast growth factor; FSH=follicle stimulating hormone; LH=luteinizing hormone; LPS=lipopolysaccharides; VEGF=vascular endothelial growth factor.

For a systematic study of protein kinase effects, Kwak and co-workers [48] used SKHep1 cells which normally express low levels of Cx45 and are not capable of Lucifer Yellow dye transfer, and transfected these cells with Cx43. The absence of dye transfer in cells only expressing Cx45 was not influenced by 8-Br-cAMP (PKA-activation), TPA (PKC activation) or 8-Br-cGMP (PKG activation). On the other hand, PKC activation by TPA favored the smaller conductance state of Cx43 channels (61 pS events), along with a decrease of the relative frequency in 89 pS events. This complicated behavior may eventually account for the diversity of results being reported in the literature. In parental non-transfected SKHep1 cells which were coupled via Cx45, activation of PKC induced an additional 16 pS conductance state (together with the 22 and 36 pS conductances observed before). However, according to Christ and Brink [58], the portion of a certain substate

contributing to the macroscopic conductance is also necessary to be considered. More recently, TPA was shown to reduce coupling in HeLa–Cx43 cells and in HeLa cells expressing both Cx43 and Cx45, but not in HeLa–Cx45 cells [59]. However, in another study HeLa cells transfected with Cx45 PKC activation increased gap junctional coupling [60]. These contradictory results may be explained by the fact that Van Veen and colleagues [60] used another phorbol ester (4-α-phorbol 12-myristate 13 acetate; PMA). Thus, it might be speculated that different PKC isoforms might have been activated. Regarding the role of cGMP, Ngezahayo and coworkers presented evidence that in GFSHR-17 granulosa cells intracellular cGMP might cause Ca^{++} entry, K^+-efflux accompanied by cell shrinkage and gap junction uncoupling, a process which might play a role in apoptotic/necrotic processes [61].

Interestingly, PKC inhibition using staurosporine (300 nM) lead to uncoupling in cultured neonatal rat cardiomocytes, as demonstrated by Saez et al. [62]. This effect could be reversed by TPA. Staurosporin in these experiments reduced the incorporation of ^{32}P into Cx43 supporting the view that PKC-dependent phosphorylation of Cx43 enhances intercellular coupling. From 2D-gel electrophoresis experiments and analysis of the phosphorylation sites Saez and colleagues [62] concluded that a protein kinase other than PKC might phosphorylate Cx43 in vivo. Another factor taken into account is the question whether Cx43 prior to treatment with TPA is in the non-phosphorylated or in the phosphorylated state. The authors concluded that Ser-368 and Ser-372 might be the targets of PKC phosphorylation in those cells which show uncoupling effects of PKC activation. However, it should be kept in mind that staurosporine in not very specific for PKC.

Angiotensin has also been shown to influence gap junction coupling in cardiac cells. The acute effects seem to be mediated via AT_1 receptors coupled to $G_{q/11}$ proteins and protein kinase C. In adult ventricular cell pairs 1 μg/ml angiotensin-II rapidly decreased g_j by 55% [63], which was reversible within 3 min. Threshold concentration was 10 nmol/l. In subsequent experiments intracellular dialysis of 10 nmol/l angiotensin I resulted in a decrease of g_j of 76%, which was completely inhibited by intracellular dialysis of 1 nmol/l enalaprilat, demonstrating the possible existence of an intracellular angiotensin converting enzyme [64]. Intracellular dialysis of angiotensin II also led to a decrease in g_j. In support of these findings renin, angiotensin I, angiotensin II and angiotensin converting enzyme have been found in cardiomyocytes using immunofluorescent staining [65]. The existence of an intracellular angiotensin receptor is also supported by a recent study [66]. Chronic exposure to angiotensin-II can alter connexin expression (see next section).

VEGF (=vascular endothelial growth factor), an angiogenetic factor, acting on tyrosine kinase-coupled VEGF-receptors (type 2) reversibly inhibits GJIC in endothelial cells within 15–30 min after application of 50 ng/ml VEGF with a concomitant change in the phosphorylation of Cx43 [67]. Besides this, a later upregulation of Cx43 (after ≥1 h) has been observed [68].

Interestingly, in a Cx43 expressing cell line, Postma and coworkers [69] described that lysophosphatidic acid, thrombin, and endothelin induced uncoupling. This could be mimicked by AlF_4^-, a direct activator of trimeric G-proteins, but was insensitive to pertussis toxin, an inhibitor of G_i-proteins. In the presence of the agonist, the coupling recovered within 1–2 h or 3–4 h (endothelin) due to receptor desensitization. The uncoupling effect was independent from Ca^{++}, PKC, MAPK, membrane potential, Rho or Ras activation, but was sensitive to tyrphostins and was not present in Src-deficient cells, so that the authors concluded that G_q-coupled receptors may uncouple Cx43-expressing cells via a Src-Tyr-kinase. G_i proteins (typically associated with muscarinic receptors such as m2 or m4) seem to regulate Cx43 trafficking and inhibition of G_i by pertussis toxin inhibits gap junction plaque formation [70]. However, carbachol in human luteinizing granulose cells leads to reduced GJIC via enhanced Cx43–Ser368 phosphorylation [71], which might – from our point of view – be explained by activation of other muscarinic receptors, such as m1, m3 or m5 which couple to $G_{q/11}$ (for review on muscarinic receptors: [72]). In rat pancreatic acinar cells (which are coupled via Cx32 and Cx26) acetylcholine in high, supraphysiological concentrations (1–5 μM) can also reduce GJIC possibly via a nitric oxide-dependent pathway [73]. In lower concentrations, it did not affect GJIC. In the same cell type, it was shown that for physiological concentrations of cholecystokinin-octapeptide (CCK-OP) (1 nM) gap junction uncoupling follows secretion [74].

Among the physiological mediators, cytokines such as IL-1β lead to reduced GJIC via Cx43 in astrocytes [75] or to a disappearance of Cx32 in rat liver cells [76]. Another cytokine, TNFα, has been shown to reduce the phosphorylated form of Cx43 in cornea fibroblasts thereby reducing GJIC. Moreover, TNFα exposure (1000 U/ml; 2–24 h) reduces GJIC and Ca^{++} wave propagation in brain endothelial cells [77]. On the other hand, TNFα in combination with interferon-γ enhanced GJIC in cultured human monocytes [78]. Regarding the effects of TNFα on Cx expression see below (third section).

Endogenous polyamines are known to accumulate at the end of the G1 phase of the cell cycle, have been suggested to stabilize DNA and tRNA, and can block a number of ion channels. Polyamines such as spermine (0.1–4.9 mM) or spermidine (>5 mM) have been successfully used for uncoupling of Cx40 channels in transfected N2A cells [79], although it is not sure whether this has a physiological function.

Nitric oxide is among the most important physiological mediators in the vasculature but also in a number of other cells. Normally the effects of NO rely on its ability to activate soluable guanylate cyclase. Besides this, nitric oxide can induce uncoupling in human umbilical vein endothelial cells (HUVEC). This effect does not seem to be mediated via cGMP [80]. It was also shown by this group that NO causes uncoupling of Cx37 coupling but increases the formation of

Cx40 containing gap junctions [81]. In a previous study, this group demonstrated increases in Cx40 membrane incorporation following application of the NO donor SNAP, which was sensitive to PKA-inhibition with H89 [82]. Although it is unclear at present how NO can activate PKA, these investigation shed light on the interesting fact that obviously NO can modulate endothelial GJIC. Uncoupling of Cx43 coupled cells, human uterine myocytes, has been previously shown by Roh and coworkers [83].

Interestingly, effects of quinones and quinolines on GJIC have also been described. The anti-malaria drug quinine, which in plasmodium is supposed to inhibit heme polymerase, can block Cx36 and Cx50 channel currents in transfected mammalian cells at 32 or 73 μM IC_{50}, respectively, but does not affect Cx26, Cx32, C40 or Cx43 channels, while Cx45 channels are moderately sensitive [84]. The underlying mechanisms for these effects still remain unknown at present. Mefloquine, an anti-malaria drug, blocks Cx36 channels (IC_{50}: 300 nM) and Cx50 channels (IC_{50}: 1.1 μM) while Cx43, Cx32 and Cx26 are less or not sensitive and can be blocked only with very high concentrations [85]. Moreover, it can potentiate the uncoupling effects of endothelin-1 or ATP in astrocytes [86]. Similarly, ilmaquinone reduces Cx43 phosphorylation (reduced Cx43-P2 band) and GJIC [87]. Increased Cx43-Ser368 phosphorylation under quinines in rat liver cells seems to involve EGF receptor activation and ERK1/2 [88]. According to recent investigations, ilmaquinone predominantly uncouples Cx43 channels within 15 min, but not Cx36, Cx45 or Cx57, while Cx26, Cx31, Cx32 channels exhibit only moderate responses [51].

In liver cell, a number of drug leading to gap junction uncoupling has been identified. Thus, the vitamin K antagonist dicoumarol (chemically a cumarine derivative; 3,3′-methylen-bis(4-hydroxy-cumarine)) uncouples Cx43 coupled hepatocytes and reduces phosphorylated Cx43 with an IC_{50} of 3 μM, while warfarin, a related compound (however, a monocumarine), is less effective (5–10 mM) [89]. Liver cell GJIC also is reduced by vitamin K, menadione, a quinone, in considerably high concentrations of 50–100 μM [90]. This effect of vitamin K and dicoumarol may indicate that the uncoupling action is not related to the vitamin K-like effect but may be linked to its chemical structure (quinone-like structures?).

A new group of drugs resulting in reversible gap junctional uncoupling comprises the fenamates or anthranile acid derivatives, which are part of the group of cyclooxygenase inhibiting non-steroidal antiphlogistics, with an order of potency meclofenamic acid > niflumic acid > flufenamic acid with IC_{50} values of 25 to 40 μM [91]. The exact molecular mechanism of this action demonstrated in SKHep1 cells expressing Cx43 remains unclear, but is not related to cyclooxygenase inhibition, PKC activation, intracellular pH, calcium or membrane depolarization. Arylaminobenzoates such as flufenamic acid can reduce the open probability of gap junction channels formed by various connexins in transected N2A cells [92]. Flufenamic acid can also be used as a hemichannel blocker in bovine corneal endothelial cells [93].

Another reversible gap junction uncoupling agent is 2-aminoethoxydiphenyl borate (normally used as IP_3-receptor blocker), which has been evaluated in normal rat kidney fibroblasts leading to uncoupling with an IC_{50} of 5.7 μM [94]. However, the underlying mechanism of action is still unknown.

Regarding toxic agents hexachlorobenzene, an epigenetic carcinogen, reduces hepatic GJIC via reduced Cx32 and Cx26 expression [95]. Among liver toxic agents, Fe overload also reduced GJIC in hepatocytes [96] as phenobarbital does [97].

Cisplatin, a cytostatic drug used in anticancer therapy, causes G1 arrest of cell cycle together with premature cell senescence and reduced GJIC in cultured human fibroblasts [98].

Lindane (hexachlorocyclohexane, normally used as insecticide) causes Ser368 phosphorylation of Cx43 and reduces GJIC in liver ands also in myometrial cells [99]. This action seems to involve oxidation of glutathione [100]. In addition, in a Sertoli cell line a redistribution of Cx43 from the membrane to the cytoplasmic perinuclear region (and similar to that of zonula occludens-1 protein) has been demonstrated under the influence of 50 μM lindane [101]. Moreover, lindane can inhibit the phosphorylation of Cx43 induced by FSH and TGFβ-1 in rat ovarian granulose cells [102].

Next, the lipophilic agents should be considered. Among lipophilic drugs, annihilative narcotics such as halothane or isoflurane also can affect intercellular coupling. Incubation of neonatal rat cardiomyocytes with 2 mM halothane resulted in a 90% reduction of initial junctional conductance within 15 s without a change in single channel conductance [103]. Subsequently, it was found that halothane reduced the mean open time while increasing the mean closed time [104]. Regarding the connexin isoforms, Cx40 channels seem to be less sensitive than Cx43 channels or channels in cells co-expressing Cx43 and Cx40 [104]. These effects may contribute to the well known arrhythmogenic effects of halothane or isoflurane.

Cardiac gap junction channels can be uncoupled using micromolar concentrations of heptanol, octanol, myristoleic acid, decaenoic acid or palmitoleic acid [105–107]. Most commonly, this is explained by an incorporation of these drugs into the lipid bilayer leading to impairment of the transcellular gap junction channels. However, oleic acid reduces GJIC via PKC-dependent Ser368 phosphorylation of Cx43 [43]. Similarly, 18β-glycyrrhetinic acid reduces Cx43 immunopositivity in the plasmamembrane [108]. The glycyrrhizic acid metabolites 18-α-glycyrrhetinic acid, 18-β-glycyrrhetinic acid and carbenoxolone have been shown to uncouple gap junction channels in various models. Thus, several authors have used 18α-glycyrrhetinic acid as a gap junction inhibitor [109] in concentrations of about 50 μM [110] or 18β-glycyrrhetinic acid in a concentration of 5 μM [111]. Glycyrrhetinic acid has been used in these studies for inhibition of intercellular communication in vascular tissue. It should be mentioned that these drugs including carbenoxolone are not specific for gap junctions. The effect of these compounds requires a longer exposure time than the above-mentioned drugs.

Heptanol has been reported to reduce coupling by reducing open probability of the channels by a conformational change at the connexin–membrane lipid interface [112]. Many investigators used heptanol which has been shown to inhibit reversibly g_j with a K_D of 0.16 mmol/l. According to Rüdisüli and Weingart [15], the uncoupling effect of heptanol is fully reversible within 2 min after washout in their experimental system (The concentration–response curve revealed a steep S-shaped relationship (Hill coefficient $z=2.3$) with a K_D: 0.16 mmol/l). Oleic acid also closes gap junctions in neonatal rat cardiomyocytes with an EC_{50} in the order of about 2 μM [105,113]. Similarly, myristoleic acid and palmitoleic acid lead to uncoupling with similar EC_{50} [105]. In whole heart Langendorff preparations of rabbit hearts palmitoleic acid exhibited a preferential impairment of transverse conduction by the fatty acid and concomitant increase in dispersion with an EC_{50} of 3.3 μM [114,107]. In that concentration range, there was no effect on the transmembrane action potential of isolated cells. The ω-6 unsaturated fatty acid arachidonic acid and precursor of eicosanoid metabolism also uncouples cells with a K_D of 4 μmol/l [115]. The concentration response curve analysis revealed a K_D of 4 μmol/l and a Hill coefficient of 0.75, and was partially reversible within 30 min. The recovery could be accelerated by addition of fatty-acid free bovine serum albumin (which binds fatty acids) to the bath solution. Since the single channel conductance was not altered by arachidonic acid in concentrations reaching 100 μmol/l, it was concluded that arachidonic acid may reduce the open probability of the channel. Interestingly, 100 μmol/l arachidonic acid did not affect non-junctional membrane currents. It should be noted, that these lipophilic drugs are not very specific for gap junctions and can inhibit other ion channels as well.

Recently, it has been shown that 11,12-epoxyeicosatrienoic acid also elicits an uncoupling effect which has been demonstrated in endothelial cells. This effect was biphasic: an initial improvement of interendothelial coupling was followed by sustained uncoupling effect which seemed to depend on activation of ERK1/2 [116]. This opens the interesting view of an endogenous intracellular regulation of intercellular communication.

Another eicosanoid reported to inhibit GJIC is thromboxane A_2. It was shown that a TXA_2 mimetic reduced dye transfer between human endothelial cells and led to internalization of Cx43 [117]. This was associated with capillary formation and thus might reflect a mechanism involved in angiogenesis.

The vascular GJIC can also be blocked using the cannabinoid receptor agonists Δ^9-tetrahydrocannabinol (10–30 μM) or the synthetic HU210 (10 μM) which both led to Cx43 phosphorylation in an ERK1/2-dependent manner associated with reduction in electrical coupling and dye transfer within 15 min in cultured endothelial cells [118].

2.3. Drugs used for acute opening of gap junctions (Table 2)

In the following those drugs should be considered which can improve GJIC within minutes. In early studies, it was found that intracellular cAMP in Purkinje fibres can enhance coupling [119–121], while in others, there is no effect [48,55]. Since in Cx32 in hepatocytes the target for PKA-dependent phosphorylation was identified as Ser-233 which is embedded in a motive (Lys–Arg–Gly–Ser) known as a consensus sequence for PKA or PKG, i.e., basic–basic-spacer-Ser and since this sequence cannot be found in Cx43, one may argue that Cx43 is not subject to direct phosphorylation by PKA. In accordance with this, Kwak and Jongsma [55] investigated the influence of 8-Br-cAMP, a direct activator of PKA, on dye coupling and electrical coupling in pairs of neonatal rat cardiac myocytes without detecting a change in coupling in response to 8-Br-cAMP. Thus, it might be that PKA activation may enhance coupling in Cx40- and Cx45-coupled cells (such as Purkinje fibres) but not in Cx43-coupled cells. This is supported by van Rijen et al. [122] showing that human Cx40 gap junction channels are modulated by cAMP in SKHep1 cells stably transfected with human Cx40 cDNA. The authors found an increase in macroscopic gap junctional conductance by 46% accompanied by a mobility shift of Cx40 protein on Western blots after application of 1 mM 8-Br-cAMP. Concomitantly, the single channel conductances changed: while without cAMP single channel conductances of 30, 80 and 120 pS were observed, after cAMP unitary conductances of 46 and 120 pS were detected. However, according to Christ and Brink [58] (as established for Cx43-coupled cells), the portion of a certain substate contributing to the macroscopic conductance has also to be taken into account.

In venular endothelial cells 8-bromo-cAMP also enhanced intercellular coupling [123], which might involve connexins Cx43, Cx40 or Cx37. Since comparable results were obtained in this study from cell lines expressing Cx43, the authors assumed a Cx43-mediated effect. On the other hand, dye transfer through Cx45 gap junction channels and electrical coupling in Cx45-transfected SKHep1 cells is not influenced by PKA activation [48], although an additional conductance state was observed. In the same model transfectants, SKHep1/Cx43 were investigated demonstrating that PKA did not influence conductance of Cx43. However, the possibility that PKA alters the open probability of the channels could not be ruled out in this study since the effects were observed in the presence of uncoupling agents. Additionally, it cannot be fully excluded that in the transfected cell line proteins necessary for the full and normal function of PKA are not expressed.

The role of PKA and cAMP in opening of gap junctions is further supported by the finding that histamine in human tonsil endothelial cells increases GJIC as a long-term effect acting via H2 receptors [32], which are known to couple to Gs and adenylylcyclase.

In addition, the bradycardiac agent tedisamil, which was developed as an antiarrhythmic, has been shown to increase gap junction conductance by 58% (0.1 μM) in cell pairs of cardiomyopathic hamsters in dependence on PKA [124]. However, it should be noted that tedisamil has been reported also to act on a number of other transmembrane ionic channels, such as sodium and potassium channels. Thus, tedisamil does not seem to be specific for gap junctions.

Another group of agents opening gap junctions are antiarrhythmic peptides. First these peptides were isolated from bovine atria in 1980 and increased synchronous beating of embryonic chick heart cell clusters was seen [125]. The structure was clarified as a hexapeptide (MW: 470; H_2N-Gly-Pro-4Hyp-Gly-Ala-Gly-COOH), which exhibited antiarrhythmic properties in various classical arrhythmia models [126] (for a detailed overview, see [127,128]). However, the mechanism of action was unclear and after a paper stating that it did not influence action potential parameters [129], the research on these peptides seemed to cease. At that time, we were interested in aspects of coupling, conduction and dispersion of action potential duration [130] and started to investigate this peptide. We initiated peptide synthesis, synthetised the natural antiarrhythmic peptide in classical Merrifield synthesis using Fmoc strategy and developed a number of chemically related peptides. We also found improved synchronization of chick embryonic cell clusters (unpublished observation) and could demonstrate that the effect of AAPnat and related peptides consists of an improvement of cellular coupling and an increase in gap junctional conductance [131–134]. The lead structure, the synthetic derivative AAP10 (H_2N-Gly-Ala-Gly-Hyp-Pro-Tyr-$CONH_2$), possesses a semicyclic structure and can bind to a membrane protein with a nanomolar K_D [41,134–136]. Since the AAP10 effect, consisting of enhancement of macroscopic gap junction conductance and Cx43 phosphorylation, was sensitive to GDP-βS (a G-protein inhibitor) and to PKC inhibitors as well as to a PKCα-specific inhibitor (CGP54345), it was concluded that AAP10 acts via a G-protein which downstream activates protein kinase Cα leading (directly or indirectly) to a phosphorylation of connexin43 resulting directly or indirectly in an improvement of gap junction conductance [41,132–134]. The putative receptor protein which binds both AAP10 and AAPnat has been detected by classical binding studies in the cell membrane [41,134,136] and – taking the chemical studies of Grover and Dhein [135,136] into account – is thought to build a cavity into which the semicyclic drug can fit. From our experiments, we assumed that the AAP10 effect is more pronounced in cells which are partially uncoupled. It has been shown that AAP10 and AAPnat did not exert other effects on cardiac tissue and did not influence the cardiac action potential [129,131]. Thus, according to our present knowledge, these peptides seem to be specific for gap junctions.

The studies of Grover and Dhein [135,136] elucidated the structure–activity relationships of antiarrhythmic peptides and revealed a semicyclic horse shoe-like structure of AAP10 (H_2N-Gly-Ala-Gly-4Hyp-Pro-Tyr-$CONH_2$) which seemed to be essential, since the cyclopeptide cAAP10RG, i.e., c(CF_3(OH)C-GAGHypPY) could mimic all effects of AAP10, while cyclopeptides with a longer or shorter bridge were inactive, or peptides which cannot form this semicyclic structure (e.g., GAGHypIY) (for more details on structure–activity relationships, see [128,136]).

A group from Zealand developed a D-amino acid AAP10-analogue ZP123 (H2N-Gly-D-Ala-Gly-D-4Hyp-D-Pro-D-Tyr-Ac), which can be applied in vivo and was shown to be stable in plasma for several days and to possess a terminal half-life time of 16 min in the rat [137]. Both AAP10 and ZP123 were effective in antagonizing second degree atrioventricular block induced by ouabain in mice [137]. Both drugs have been shown to reduce dispersion of action potential duration in a 256-electrode mapping in isolated rabbit hearts [138], which was not seen in the study by Kjolbye et al. [137] probably due to the small number of electrodes (8 electrodes). As AAP10 or AAPnat ZP123 also activates protein kinase C [138] and slowly increases coupling [139] as was described for AAP10 [41].

Among the lipophilic drugs and fatty acids, the polyunsaturated ω-3 fatty acid eicosapentaenoic acid (10 μM) has been assumed to enhance or preserve gap junctional coupling in human endothelial cells submitted to hypoxia/reoxygenation, probably via antagonizing free radical effects [140]. Hypoxia/reoxygenation reduced GJIC in these cells after 2 h of reoxygenation, which could be inhibited by a 2 days pretreatment with 3 μM eicosapentaenoic acid [141]. Eicosapentaenoic acid in these experiments inhibited tyrosine phosphorylation of Cx43 induced by hypoxia/reoxygenation, while under normoxia, the drug had no effect on GJIC.

In the vasculature hyperpolarization and vasorelaxation can be conducted along the vessel via interendothelial gap junctions. In some species, hyperpolarization and vasorelaxation, conducted along the vessel via interendothelial gap junctions [142,143], seem to be linked to NO/PGI_2-independent pathway coupled to the cytochrome P450 isoform CYP2C and the generation of epoxyeicosatrienoic acids such as 11,12-epoxyeicosatrienoic acid (11,12-EET) [116]. 11,12-EET (3 μM) had a biphasic effect on GJIC in human umbilical vein endothelial cells as assessed by Lucifer Yellow dye transfer and double cell voltage clamp. 11,12-EET transiently enhanced GJIC within 1 min in a PKA-dependent manner, followed by a prolonged uncoupling effect. Since EETs are potent intracellular mediators and are involved in several signal transduction cascades, these observations might be of general interest. Moreover, in vascular smooth muscle improvement of GJIC by 5-hydroxytryptamine has been described [144].

Finally, a sulfur compound from garlic, diallyl-disulfide (1–50 μM) time-dependently enhances GJIC via Cx43 in rat liver epithelial cells [145].

2.4. Drugs used for regulation of connexin synthesis, trafficking and degradation (Table 3)

In the last years, there is accumulating evidence that GJIC in many cases is regulated via enhanced or depressed expression of connexins, or by alteration of the gap junction density in the membrane. A number of drugs which enhance connexin expression or gap junction density in the membrane and GJIC have been shown to exert anti-cancer effect and to reduce tumor growth.

Thus, kaempferol, a flavonol and potential anti-cancer drug, has been shown to enhance Cx43 expression and phosphorylation in colon cancer cells [146]. Kaempferol restored differentiation in partially differentiated cancer cells but was

ineffective in undifferentiated cells devoid of Cx43 expression. Among the miscellaneous agents enhancing GJIC, the ethanol extract of Indian medical herb psyllium has been described to reduce tumor growth together with increases in GJIC in WB-Ha-ras tumor cells [147,148]. This action seems to rely on β-sitosterol contained in psyllium and an increase in Cx43 protein [147].

Carotenoids and retinoids also enhance Cx43 expression and inhibit tumor growth by a G1 arrest, which may be related to their anti-cancer efficacy (prevention) [149]. Thus, 24 h incubation of rat C6 glioma cells with 1–10 μM all-trans retinoic acid increases GJIC, but does not affect Cx43mRNA so that the authors assumed a posttranslational regulation [150]. On the other hand, 3–7 μM lycopene, a terpene serving as a precursor of β-carotene, reported to reduce cancer risk, reduces tumor cell growth and increases Cx43 protein and Cx43 mRNA in human oral cavity tumor KB-1 cells [151]. TAC-101, an agonist at retinoic acid receptors, increased Cx43 expression without a change in phosphorylation and prevented from inadequate Cx43 localisation in canine kidney cells treated with the carcinogens $KBrO_3$ and dimethylnitrosamine [152]. Consequently, the impairment of GJIC under the influence of the carcinogens was inhibited by TAC-101 in that study. Similarly, the H_2O-soluable carotenoid Na_2-disuccinate astaxanthine increases both Cx43 expression and gap junction formation thereby improving GJIC [153]. However, although β-carotene is known to enhance GJIC and Cx43 expression, the clinical in vivo situation is more complex: in clinical trials, additional dietary β-carotene increased lung cancer incidence. High doses of the drug (50 mg/kg/day) also reduced GJIC in rat liver. Interestingly, the oxidized form of β-carotene, which might be generated in in vivo metabolism, inhibits GJIC at concentrations of 5 μM in human A549 lung cancer cells [154], a result which underlines the importance of the transfer of the cell culture-based gap junction research to in vivo or isolated organ disease models. Another example is indole-3-carbinal, a natural anti-cancer agent, which, however, is converted to indolo[3,2-b]carbazole in the stomach at acid pH. Incubation of WB-F344 rat hepatocytes with 0.1–1 μM of this derivative for 8–12 h leads to reduced GJIC and loss of Cx32 expression [155]. Indolo[3,2-b]carbazole is known as a tumor promoting agent. The loss of GJIC has been linked to tumor progression (see [156]) as was also recently shown for the reduction in Cx26, Cx32 and GJIC in endometrial carcinoma cells (IK-ER1, overexpressing the ERα-receptor) by 17β-estradiol acting via ERα-receptors [157]. Several non-genotoxic carcinogens are considered to impair the balance between cell growth and cell death. This balance is often considered to be influenced by GJIC, since growth regulatory signals can be exchanged between the cells via gap junction channels and reduction in GJIC, e.g., by loss of gap junction plaques, has been shown to play a role in the cancer process. According to these considerations, typical non-genotoxic carcinogens such as Wy-14,643, methapyrilene, hexachlorobenzene and 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), chloroform and p-dichlorobenzene reduced the expression of Cx32 gap junction plaques in liver and kidney of rats treated with these drugs for 3 or 28 days [158]. Although this was not correlated with the induction of cell proliferation, the authors nevertheless concluded that this reduction in GJIC might be important in the cancer process, if such a non-genotoxic carcinogen and this reduction in GJIC occurs together with a proliferative stimulus (or perhaps a terminal carcinogen). Among the epigenetic tumor promoters, polychlorinated biphenyls (PCB) are effective in two stage cancer models. The non-planar PCB have been shown to act as potent inhibitors of GJIC while the co-planar PCB did not exert an effect on this parameter [159].

Phellinus linteus extract, derived from a mushroom considered a natural anticancer agent, has been shown to block the p38 / ERK1/2 MAPK-mediated downregulation of Cx43–GJIC and the hyper-phosphorylation of Cx43 in rat liver epithelial cells [160]. Activation of p38 MAPK (by anisomycin) was shown to result in downregulation of Cx32 in rat hepatocytes [161].

An interesting additional aspect comes from a recent study by Chen et al. [162] showing that in lung cancer cells (H2170 cells), the expression of Cx26 is reduced and that exposure to the de-methylating agent 5-aza-deoxycytidine can lead to a Cx26 re-expression, so that the authors concluded that the reduction in Cx26 might be a consequence of Cx26 promotor methylation. On the other, the anti-proliferative effects of increased Cx43 expression should also be considered on the background that the carboxy terminal of Cx43 (at least of this connexin) suppresses growth, e.g., of N2A cells [163].

Besides cancer induction and anti-cancer treatment, pharmacological interference with gap junction expression has been shown to affect neurological function or disorders like seizure. Thus, bicuculline methiodide (a GABA(A) receptor antagonist; 10 μM, 18 h) induces epilepsy-like discharges in cultured hippocampal slices together with increased GJIC (indirectly measured) and an increase in Cx43 and Cx32 protein and mRNA levels as well as expression of the transcription factor c-*fos*, while Cx26 and Cx36 were not affected. The epilepsy-like discharges and GJIC in that model could be blocked by the gap junction blocking agent carbenoxolone [164].

Moreover, amphetamine withdrawal (in vivo, rat model of amphetamine addiction) results in reduced neuronal Cx36 expression in rat nucleus accumbens and prefrontal cortex, which are areas known to be involved in the mechanism of addiction [165].

Cortical astrocytes are coupled via Cx43. The Cx43 protein and mRNA level can be reduced by epidermal growth factor (EGF) in a MEK-dependent manner [166] (EGF is known to induce hyperphosphorylation, ubiquitination and internalization of Cx43 [167,168]). The level of Cx43 in these cells might be important for the propagation of cell death during ischemia as was elegantly shown by Contreras et al. [169] by inhibition of GJIC in an astrocyte ischemia-model. Recently, it was also demonstrated that endothelin can reduce expression of the phosphorylated isoform of Cx43 and diminish GJIC in cultured astrocytes [170].

Regarding the action of hormones, in the promoter region of the Cx43 gene, a series of half-palindromic estrogen response elements has been identified using a luciferase reporter [171].

In the following years, investigators have tried to find hormonal regulation of connexin expression. Thus, estradiol enhances Cx26 together with a reduction in clusterin in rat endometrium under in vivo conditions. The effect could be inhibited by tamoxifen, raloxifen or diethylstilbestrol [172]. The effect on Cx26 is in contrast to that described by Saito et al. [157]; however, the latter study was carried out in endometrial cancer cells overexpressing ERα-receptors, so that one might imagine that different signal transduction pathways may be involved. Regarding Cx43, estradiol had no effect on Cx43 in rat endometrium under in vivo conditions [172]. In addition, 17β-estradiol (EC_{50}: 400 nM) has been shown to counteract the Ser-368-phosphorylation of Cx43 and reduction in GJIC induced by metabolic inhibition in rat neonatal cardiomyocytes [173]. Liu et al. [174] showed that in ovariectomized female Wistar rats, Cx43 was significantly downregulated in media and endothelium of mesenteric arteries associated with a decreased EDHF response. Since this could both be normalized by 17β-estradiol, estrogen seems to be involved in the regulation of Cx43 in the vasculature (at least in this model) in endothelial and myoendothelial gap junctions. The functional interplay between the components of the ovarian follicle is subject to hormonal regulation and is also controlled by GJIC. The most abundant connexin in the follicle is Cx43. FSH upregulates Cx43, while LH downregulates Cx43 levels. This LH-induced downregulation is due to a reduced rate of translation in a PKA and MAPK dependent manner [175].

Finally, thyroid hormone receptors can bind to an element in the Cx43 promotor identified at position −480 to −464 [176]. However, an increase in Cx43mRNA following thyroid hormone treatment of Wistar rats was only seen in liver cells, while in heart cells, there was no change in that study. However, in neonatal rat cardiomyocytes, the thyroid hormone T3 was shown to enhance Cx43 [177].

Another interesting aspect of chronic regulation of gap junction expression involves the role of HMG-CoA-reductase inhibitors, statins and low density lipoproteins (LDL) in the formation and stabilization of atherosclerotic plaques. Thus, after arterial lesions together with cholesterol-rich diet, an upregulation of Cx43 has been observed in smooth muscle cells in particular in the primary intimal layer, while it was reduced in the media [178]. Cx43 was not found in the macrophages in that study. In mice, a cholesterol-rich diet led to downregulation of Cx37 and Cx40 in aortic endothelial cells. Interestingly, the Cx37 decrease could be reversed by simvastatin treatment, while the Cx40 downregulation could not [179]. Moreover, in the vasculature of LDL-receptor-deficient mice fed on a cholesterol-rich diet, the vascular atheromas exhibited thicker fibrous caps (which would decrease the risk of dissection) and less inflammatory cell infiltration together with reduced Cx43 expression, if these mice were treated with a HMG-CoA-reductase inhibitor (pravastatin) [180]. In human vascular cells, it was shown that HMG-CoA-reductase inhibitors (statins) can reduce Cx43 expression by a yet unknown mechanism [180]. On the other hand, it was shown that nicotine can downregulate the expression of Cx43 in human umbilical vein endothelial cells, and that this effect can be attenuated by a number of statins (fluvastatin, lovastatin, pravastatin, simvastatin) probably independent from the mevalonate metabolism [181]. Thus, among the various pleiotropic effects of statins, a modulatory action on the expression of connexins has also to be considered, which might contribute to the antiatherosclerotic action of these drugs (probably with divergent actions in the cell types involved (endothelium, neointimal cells, vascular smooth muscle cells, etc.).

Several transcription factors have been identified to play a role in the regulation of connexin expression. Regarding transcriptional control of Cx43 and Cx40, the promotor regions of Cx40 gene [182] and of Cx43 gene [183] contain an AP-1 Site. Additionally, a TATA box, an AP-2 site and an estrogen-responsive element have been found [171] (see also above). Moreover, the T-box transcription factor Tbx5 seems to be involved in Cx40 regulation, since heterozygous Tbx5(del/+) mice exhibited a marked downregulation of Cx40 [184].

The homeodomain-containing transcription factor Csx/NKx2.5 (a member of the NK2-class homeodomain protein and an early cardiogenic marker) may also be involved in the regulation of Cx43 and Cx40. Mutation of the Csx/Nkx2.5 (I183P) in mice led to a marked downregulation of both connexins [185]. The authors concluded that these connexins may be direct or indirect downstream targets of Csx/NKx2.5. Similar mutations have been detected in patients suffering from congenital atrioventricular conduction defects. In support of these findings, several consensus binding sites for Csx/NKx2.5 (TNAAGTG) [186] were found within the Cx40 and Cx43 promotors [182,187]. In addition, two Sp1/Sp3 binding sites have been identified in the promotor of the rat Cx40 gene contributing to the transcriptional activation of this gene in cultured cells [188].

Since the gene structure of connexins is known, it is also possible to inhibit gap junction expression specifically by the delivery of antisense oligonucleotides. Thus, using (1994)5′-GTCACCCATGTCTGGGCA-3′ as Cx43 antisense and 5′-GTCACCCATCTTGCCAAG-3′ as Cx40 antisense in A7r5 cell (embryonic rat aorta smooth muscle cell line expressing both Cx43 and Cx40) Moore and Burt could show, that 24 h treatment with one of the oligonucleotides (30 μM in cell culture medium) could suppress the unitary conductances specific for the target connexin [189]. This is, however, at least at present only of experimental importance and not for therapeutic use.

Regarding the regulation of connexin synthesis by classical signal transduction pathways it has been shown that connexin expression can be induced by the second messenger cAMP [190]. Darrow and colleagues [190] found an upregulation of Cx43 and Cx45 after 24 h treatment with dibutyryl cAMP in cardiomyocytes. Accordingly, Salameh and colleagues [191,192] showed an upregulation of Cx43 in neonatal cardiomyocytes in response to forskolin, a direct activator of adenylylcyclase. Thus, there is evidence that activation of the adenylylcyclase/ cAMP /PKA pathway can enhance Cx43 expression. Because in the cardiovascular system this pathway

typically is activated by β-adrenergic stimulation, this mechanism might play an important role in the process of adaptation of the heart to increased stress and higher heart rates. Moreover, other factors stimulating adenylylcyclase as well such as, e.g., prostaglandins might also have an effect on Cx43 expression. Besides in cardiomyocytes, cAMP also induces synthesis of Cx43 in fibroblasts as was shown using the stable analogue 8-Br-cAMP. Since this effect could be suppressed by H89, it was concluded that the cAMP-effect on Cx43 synthesis is mediated via PKA [193]. In that study, the role of serine 364 for the PKA-dependent effect was elucidated.

Gq/11-coupled receptor activation, typically leading to PKC and MAPK activation (ERK1/2 or p38), also can upregulate Cx43 synthesis as was shown in neonatal rat cardiomyocytes by 24 h endothelin-1 exposure (10–1000 nM, each concentration applied for 24 h) which lead to an increase in Cx43 expression (EC_{50}:158±41 nM) and phosphorylation (EC_{50}: 13±3 nM) while Cx40 remained unaffected [194]. The increase in Cx43 was reflected by enhanced gap junctional conductance in double cell patch clamp experiments after 24 h endothelin treatment after wash-out and in absence of endothelin. The endothelin-effect was mediated via ET_A-receptors, since it could be antagonized by BQ123 (an ET_A-receptor antagonist), but not by BQ788 (an ET_B-receptor antagonist). Downstream, it was found that the enhanced Cx43 expression was dependent on ERK1/2 [194]. Similarly, angiotensin-II, another agonist acting at $G_{q/11}$-coupled receptors, also concentration-dependently can increase the expression of Cx43 [195]. In a subsequent detailed pharmacological study 24 h exposure to angiotensin-II the treatment lead to enhanced Cx43 protein levels (EC_{50}: 57±10 nM) and phosphorylation (EC_{50}: 93±8 nM) and increased GJIC (10–1000 nM, each concentration was applied for 24 h; electrophysiological tests after thorough wash-out), while – as with endothelin – there was no detectable change of Cx40 [194]. The angiotensin-II-induced Cx43 expression was mediated via the AT_1-receptor because of its sensitivity to losartan, an AT_1 receptor antagonist [194]. Regarding the signal transduction pathway, it was demonstrated that angiotensin activates both ERK1/2 and p38 signal pathway [194]. Enhanced Cx43 expression via enhanced biosynthesis under the influence of angiotensin-II was also observed in rat liver cells [196]. Interestingly, an antagonization of stretch-induced augmentation in Cx43 expression by the AT_1-receptor antagonist losartan has also been described supporting a role for angiotensin-II mediated mechanisms in stretch-dependent regulation of connexins [197].

Besides angiotensin-II, VEGF seems to play an important role since Pimentel and colleagues [68] also found increased Cx43 and conduction in cultures of neonatal rat cardiomyocytes upon stretch in association with VEGF-secretion. This stretch-induced enhancement of conduction could be antagonized by a VEGF antibody and by a TGF-β antibody indicating that stretch might induce increase in Cx43 expression via a TGF-β /VEGF pathway [68].

In cardiac remodelling processes, basic fibroblast growth factor (bFGF) plays an important role. bFGF has been shown to induce Cx43 expression in cardiac fibroblasts within 6 h after administration associated with enhanced GJIC assessed by scrape load technique [198]. Such regulation of intercellular fibroblast communication might play a role in arrhythmogenesis in cardiac fibrosis. Interestingly, in cardiomyocytes bFGF exposure acutely (within 30 min) decreased gap junctional coupling in a PKCε-dependent mechanism (see above) [42]. Unfortunately, there are at present no data available on the effects of chronic (≥24 h) bFGF stimulation of cardiomyocytes.

In addition to the above-mentioned factors, cytokines may also play a role in the chronic regulation of connexin expression. In endocardial biopsies from heart transplant recipients decreased Cx43 expression was found during acute cellular rejection [199], indicating that cardiac allograft rejection can lead to downregulation of Cx43. Among the factors that could play a role in this pathophysiology, cytokines and tumor necrosis factor α (TNFα) should be considered. TNFα transduces its effects via TNFα-receptor associated factors (TRAF1-6) and simultaneous activation of NF-kB, JNK and p38 MAP-kinase. With regard to the TNFα-effect on connexin expression, there are diverging results reported in the literature: in bacterial lipopolysaccharide (LPS)-induced cardiac inflammation in hearts in an in vivo rat model, Fernandez-Cobo et al. [200] found a downregulation of Cx43–mRNA, while in contrast, in lung and kidney, Cx43 was increased following LPS-exposure [201] as was also found in liver [202]. In H9c2-cells (transfected with the Cx43-promotor), the Cx43 promotor activity could also be reduced by incubation with lipopolysaccharides and to a similar extent with TNFα (2–500 ng/ml) [200]. In contrast, in cultured neonatal rat cardiomyocytes, 24 h exposure to low concentrations of TNFα (10 U/ml) increased Cx43 expression via p38 MAP-kinase [203,204]. This is in good accordance with the findings by McLaughlin and colleagues [205] who demonstrated that TNFα can stimulate MAPKAP kinase 3, which is a substrate of p38 MAP-kinase. In support of these data, Klein et al. [206] showed that TNFα can indeed cause phosphorylation of p38 MAP-kinase. Moreover, TNFα also increased Cx43 expression in microglia cells if TNFα was applied together with interferon-gamma, while it did not influence Cx43 if applied alone [207]. On the other hand, in endothelial cells, 0.5 nM TNFα did not influence Cx43 expression but led to downregulation of Cx40 and Cx37 [208]. Thus, in different cell types, obviously different signalling pathways connected to Cx43 regulation are activated in response to TNFα-receptor stimulation (it should be noted that the TNF receptor family comprises 18 receptors). It has further to be taken into account that higher concentrations of TNFα also can induce apoptosis and cell death, which might also influence gene transcription. Regarding the septic shock model with LPS-exposure, many other factors and complex hemodynamic changes may play a role as well.

Besides these receptor-mediated pathways, other agents might affect connexin synthesis. Thus, ethanol reduces the Cx43 biosynthesis in liver cells [196]. Together with reduced GJIC, this was also seen in P19 cells using a concentration of 20 mM ethanol (approximately 0.1%) [209].

Regarding the assembly of Cx43 gap junction channels, assembly seems to be regulated by the interaction of Cx43 and zonula occludens ZO-1 protein [6]. Interestingly, it has been found that c-Src, a tyrosine kinase which can phosphorylate tyrosine residues of the Cx43 protein, leads to closure of Cx43 gap junctional channels via disrupting the interaction of ZO-1 and Cx43 involving tyrosine-phosphorylation of Cx43 [210]. This mechanism might play a role in the pathophysiology of heart failure, since in cells from cardiomyopathic hamsters increased c-Src activity was found correlated with increased tyrosine phosphorylation and reduced gap junctional communication [211].

A number of substances and mediators have been shown to interfere with the synthesis, formation, docking and degradation of the connexins. After synthesis, connexins are transported to the Golgi apparatus, oligomerized and thereafter transported to the membrane [4]. It is possible to inhibit the transport to the Golgi apparatus by brefeldin A as shown in a very elegant study for Cx43 in cultured rat kidney cells [4]. Connexin can be trapped within the *trans*-Golgi network by the metabolic inhibitor monensin [212]. Since connexins co-localise with other proteins, regulation of these proteins is also important to consider. In that regard, wnt-1 signalling has been investigated. Wnt-1, a protooncogene encoding a secreted developmental protein, was reported to result in increased Cx43 transcription and accumulation of Cx43 co-localised with β-catenin [213]. This was accompanied by enhanced coupling. Since wnt-1 signalling regulates a variety of cells and developmental processes, the influence of wnt-1 on Cx43 might be an interesting new aspect of Cx43 regulation.

Connexons dock to each other forming the complete gap junction channel by interaction of their extracellular loops. Antibodies raised against these extracellular domains have been shown to inhibit gap junction assembly. From a pharmacological point of view, it should be possible to interfere with the docking process by adding peptide sequences resembling only the extracellular loops as first described for Cx32 [214] using them as a kind of competitive inhibitors. In experiments using chick cardiomyocytes, the motifs QPG and SHVR in extracellular loop 1 and SRPTEK in loop 2 were identified [215]. In rabbit ear arteries, the peptides GAP27 and GAP26 were used as inhibitors of GJIC formed by Cx43 and Cx37 [216,217]. GAP26 has been reported to act primarily on hemichannels [218] and may inhibit ATP release. A peptide-analogoue of the second extracellular loop of Cx43, P180–195 (SLSAVYTCKRDPCPHQ; 500 μM) in A7r5 smooth muscle cells inhibits coupling via Cx43 channels and a second peptide, analogue to the second extracellular loop of Cx40, P177–192 (FLDTLHVCRRSPCPHP; 50 μM, higher concentrations could not be used due to lower soluability) blocked coupling via Cx40 channels [219]. Kwak and Jongsma [219] showed that 24 h treatment of the cultured cells was sufficient to suppress the portion of intercellular coupling which could be ascribed to the target connexins by suppression of the unitary conductance specific for the target connexin. A peptide homologous to the carboxy terminus was used as control peptide and did not affect coupling. Another peptide often used for inhibition of docking is also analogous to the extracellular loop of Cx43, called the 43GAP27 peptide (SRPTEKTIFII). In a concentration of 300 μM, it was shown to inhibit the endothelial component of cannabinoid-induced relaxation in rabbit mesenteric artery [110]. Moreover, acetylcholine-induced relaxation in rabbit central ear artery could be antagonized by 300 μM GAP27. However, the effect varied with the branching of the artery and was more prominent in vessels of the second generation [220].

The last step in the life of gap junctions is the degradation of connexins, which also can be modulated pharmacologically. Connexins can be degraded either by the proteasomal pathway or via the lysosomal pathway. Connexins can be degraded via the lysosomal pathway after internalization (so called anular gap junctions). Alternatively connexins can be degraded via the ER-associated proteasome pathway (ER = endoplasmic reticulum), which seems to be the prominent pathway for misfolded or improperly oligomerized connexins [221]. Parts of the degradation of Cx43 seem to be dependent on prior ubiquitinylation of the protein. The lysosomal pathway can be inhibited by protease inhibitors such as leupeptin or by disruption of the pH gradient in the lysosome using chloroquine, primaquine or balifomycin A, leading to enhanced Cx43 presence in the cells [222]. The proteasome can be inhibited by ALLN (acetyl–leucyl–leucyl–norleucinal), lactacystin, clastolactacystin and epoxomicin [223]. These agents have been widely used to study gap junction degradation, but there is no therapeutic approach so far. Interestingly, Laing et al. [222] showed that heat stress led to reduced Cx43 expression, which could be prevented by lactacystin, ALLN and chloroquine. These authors concluded that heat stress may mimic other stress, such as ischemia, which also is known to reduce Cx43 expression.

2.5. Therapeutic perspectives and conclusions

There are a number of possible indications for of gap junction affecting drugs including bone fracture, immune system, heart disease, arrhythmia, atherosclerosis, learning, seizure, regulation of secretion, female infertility, cancer, inflammation and stem cell therapy. A number of drugs is available affecting gap junctions on various levels. Problems with these drugs still are specificity for a certain connexin or organ and subcellular distribution/geometry, i.e., the question whether a simple upregulation of a connexins leads to the correct insertion and localisation of the newly formed gap junctions.

Most studies addressing the pharmacology of gap junctions are related to cell culture models. At present, there is a striking lack of studies clearly demonstrating a beneficial effect of a gap junction affecting drug in a disease model (in vivo or isolated organ) or even in humans. This needs to be the next future step in the chapter of pharmacology of gap junctions.

Seizure at a first glance is a result of abnormal excessive synchronized electrical activity of large groups of neurons, which is considered to be the consequence of disturbances of the intracellular homeostasis of the cells, or of an imbalance between excitatory and inhibitory activity. However, the

abnormal activity of a small group of cells has to spread in order to involve larger cell groups thereby generating the typical picture of a generalized seizure. This spreading has been considered previously to be due to synaptical contacts between the cells involved. However, in the past years, it was found that cells of the nervous system (neurons and glia cells) also exhibit gap junctions allowing the transfer of electrical activity and establish a kind of "gap junction wiring" [224]. Thus, in rat central nervous system, Cx26, Cx30, Cx32, Cx36 and Cx43 has been detected. Oligodendrocytes express solely Cx32, astrocytes Cx26, Cx30 and Cx43, ependymocytes only Cx43, leptomeningeal cells Cx26 and Cx43, while neurons express exclusively Cx36 in this species forming a highly complex network [225]. From these findings, the hypothesis can be formed that the generalization or spreading of seizure-like activity might also involve gap junction coupling. In support of this view, it was shown that seizure seems to be sensitive to modulation of GJIC. Thus, seizure-like activity in CA1 hippocampal region evoked by alkalization can be suppressed by agents known to block gap junction (although not specific) like carbenoxolone, sodium propionate and octanol [226], indicating a possible anticonvulsant action of gap junction blockers. In the future, brain-specific gap junction modulator drugs would be needed, which possess pharmacokinetic properties allowing the drug to pass the blood–brain barrier.

In the heart, cardiac myocytes also form an electrical network being in contact via gap junctions which are located mainly at the cell poles, while at the lateral borders, there is no or only minor expression of gap junctions. Together with the elongated cell morphology, this forms the basis for the anisotropic properties of the cardiac tissue with lower resistance in longitudinal direction than in transverse (referred to the fibre axis). This organization allows a regular spreading of the action potential. The impulse normally is generated in the sinus node (only very scarce expression of Cx43 at the interface to the atrium), spreads over the atrium (Cx40 > Cx43 > Cx45) to the atrioventricular node (sparse expression of Cx40 >>> Cx43 >> Cx45), from where after delay, it passes to the specific conduction system (Cx40 >>> Cx43 >> Cx45) and finally reaches the ventricular myocardium (Cx43 >> Cx45) (for review, see [29,227,228]. Arrhythmia is a common and serious problem in cardiovascular medicine involving supraventricular and ventricular arrhythmia which may be tachyarrhythmic or bradyarrhythmic. Theoretically, gap junctions may contribute to arrhythmia either by closure, e.g., during acute myocardial infarction (due to a plethora of effects including drop in pH, loss of ATP, Ca^{++} and Na^{+} overload and accumulation of long chain acylcarnitines), or downregulation (this, however, would probably means a very drastic downregulation of more than 90% (see [229])) leading to a conduction deficit, or by disturbances in their distribution within the tissue or within the cell (no longer confined to the cell poles as was described for atrial fibrillation [230]), thereby alterating the biophysics of the tissue. On the other hand, the balance between current source and sink is import to allow successful propagation (see [231]). According to these investigations, propagation can fail if a small group of depolarizing cells ("source") is well coupled to a yet unpolarized large area of cells ("sink"), since too much current is lost to a too large area so that the current density is not high enough to allow depolarization above the threshold resulting in conduction failure [231]. Thus, loss of coupling, but on the other hand, enhanced coupling both can – depending on the situation – cause conduction failure. The involvement of gap junctions in various forms of arrhythmia is still a matter of debate. However, several models support the role of gap junction uncoupling in arrhythmogenesis. Thus, atrioventricular conduction disturbances were shown in Cx40 knock-out mice [232], while Cx43 knock-out is letal so that only heterozygous Cx43+/− could be investigated which did not show ventricular conduction failure [233], although the incidence of inducible ventricular fibrillation was enhanced in one study [234] (for a recent review on genetic models of connexin function in the heart, see [228]). Pharmacological models, however, show transverse conduction slowing and failure if gap junction uncoupling drugs (heptanol, palmitoleic acid) are applied [106,107]. Thus, regarding pathological electrical activation, cardiac arrhythmia may be a target for gap junction modulating drugs in some (but not all) forms of arrhythmia [127]. Thus, the antiarrhythmic efficacy of antiarrhythmic peptides has been shown in various models of acute arrhythmia ($CaCl_2$, aconitine, ischemia–reperfusion and ouabain) with some of these peptides [131,137,139,235,236]. In addition, it was shown that AAP10 enhances coupling and reduces dispersion (regional inhomogeneity of action potential duration), which typically predisposes for the occurrence of reentrant arrhythmia [131]. Moreover, Xing et al. [139] found that the AAP10-analogue ZP123 (which also reduces dispersion [138]) prevented from reentrant ventricular tachycardia at plasma concentrations ranging from 1 to 69 nM in a dog model of reproducible infarct-induced tachycardia.

In persisting heart disease, a special problem is the alteration in the distribution of connexins with regional and subcellular alterations and inhomogeneities (e.g., in atrial fibrillation [230,237]). Moreover, the expression of connexins and the ratio between Cx43 and Cx40 (and sometimes Cx45) can be altered [238]. Such inhomogeneities in connexin distribution, lateralization of connexins and alterations of the expression level of a certain isoform may stabilize the arrhythmogenic substrate. Thus, in these situations (e.g., chronic heart failure; chronic phase of myocardial infarction; persisting atrial fibrillation), a simple opening or closure of gap junctions may be ineffective, and ways have to be detected to modulate the biosynthesis and the correct localisation of the newly formed connexins. However, it must also be stated clearly that other factors also contribute to the arrhythmogenic substrate such as age-dependent or atrial fibrillation-dependent increase in fibrosis with mostly lateral accumulation of collagen and in consequence transverse uncoupling [114,239].

In the cardiovascular field, hypertension and atherosclerosis are among the most common problems in Western societies. These diseases have a very complex pathophysiology which to discuss is beyond the scope of this review. However, GJIC also

is involved in some aspects of both diseases. Within the vascular wall, Cx37, Cx40 and Cx43 are expressed in the endothelial layer (intima), Cx43 and Cx45 in the media layer (although Cx37 and Cx40 have also been described in certain vessels [240]. The distribution of isoforms may vary depending on the type of vessel and its position in the vascular tree [240]. There is communication within a layer (intima or media) along the vessels' axis and between the two layers [241,242].

Thus, GJIC plays also a role in the regulation of vascular tone (see above) and has been shown to be involved in vascular relaxation by the endothelium-derived hyperpolarizing factor EDHF [109]. Rhythmic contractions of arteries (rabbit ear arteries) also involve GJIC [216]. Moreover, it was demonstrated that the up-stream vascular tone regulation is dependent on GJIC [243]. A role for GJIC in the (complex) pathophysiology of hypertension is supported by the finding that Cx40 knock-out mice suffer from hypertension [244] and that Cx43 expression is upregulated in the smooth muscle aortic cells of chronically hypertensive rats [240]. These observations indicate that a modulation of GJIC may affect blood pressure, although until today, there is no study demonstrating a clear antihypertensive effect by gap junction modulation.

Regarding atherosclerosis, it was found that the expression of connexins is altered within the atheroma [245–247]. According to these authors, monocytes invading the atheroma mature to macrophages expressing Cx37, while smooth muscle cells migrate to the intima exhibiting increased Cx43 expression in early atheroma. In this state, lipids start to accumulate in the extracellular space and in the intimal smooth muscle cells. Connexin expression in the endothelial cells and the media is not altered. However, in advanced atheroma, a fibrous cap is formed by intimal smooth muscle cells and extracellular matrix and diseased endothelia cells. Cx43 in intimal smooth muscle cells is downregulated at that stage and in the diseased endothelial cells connexin expression is almost absent (cap), while at the shoulder of the atheroma in that advanced stage the endothelium expresses Cx43 [247]. One might image that the lack of connexins in the cap region may destabilize the plaque and make it more prone to dissection. Interestingly, it was observed that statin therapy can upregulate Cx expression in atherosclerosis [179,181] (and see above). This might be linked to the observation that statins can stabilize atherosclerotic plaques. In summary, GJIC plays a role in recruitment and invasion of leukocytes in atherosclerosis as well as in formation of the plaque [247], although this is probably not a causal role, but may offer new possibilities to interfere with. Thus, both hypertension and atherosclerosis might open possibilities for gap junction modulators.

Cancer and anti-cancer therapy still is among the most important problems in medicine. First of all, it is necessary to understand the process of cancerogenesis, tumor initiation and progression: the hallmarks of cancer according to Hanahan and Weinberg [248], the self-sufficiency in growth signals, insensivity to growth inhibitory signals, evasion of apoptosis, limitedless replicative potential, sustained angiogenesis and tissue invasion and metastasis. Thus, there is initiation of a cell, promotion and thereafter invasive growth. It has been hypothetized that at least in some cases, tissue stem cells and early progenitor cells are the targets of the initiation event. Since these cells are naturally immortal and become mortal after differentiation, a cancerogenic process has to prevent mortalization of these cells [249]. Three critical epigenetic events contribute to cancerogenesis: inhibition of apoptosis, disruption of communication resulting in a loss of growth control by the neighboring cells [250] and activation of a mitogenic pathway [159]. It was Loewenstein as early as 1966 [251] formulating that GJIC is associated with growth control and differentiation in normal cells and – in consequence – that cancer cell loose their ability to communicate with the neighboring cells via gap junctions. There is a large body of evidence that GJIC is involved in cancer promotion (by epigenetic cancerogens/promoters or other factors) [for review, see [252]. Thus, an important therapeutic indication for gap junction modulating drugs might be the modulation of GJIC in particular by affecting the biosynthesis of connexins in anticancer therapy [156]. In principle, enhanced GJIC can restore growth control of a cell by its neighbors and the balance between cell growth and cell death. Most tumor cells show reduced (or no) connexin expression and, thus, re-expression of connexins and restoration of functional communication between the cells has been shown to reduce tumor cell growth. The anti-growth potential of carotenoids has been related to their ability to enhance GJIC [150–155] and a number of studies, which not all can be cited here, indicate a tumor-suppressing role for drugs which enhance GJIC [150–160]. However, to evaluate the clinical importance of these data (see [156] and above), it is necessary to test this therapeutic approach in in vivo cancer models.

In particular, after metastasation, this therapeutic approach might reach its limits since it only works if the connexins of the tumor cell (being influenced by the treatment) and those of the surrounding cells (recipient tissue, where the metastasis is) can form functional gap junction channels, which is not possible between all connexin-isoforms.

In conclusion, modulation of GJIC may open interesting new therapeutic approaches for a large number of diseases since intercellular communication in many organs plays a vital role. However, in many respects, we have not completely understood the complex networking and its long-term regulation. This again underlines the need for in vivo and organ-based models to evaluate the possibilities of pharmacological approaches towards an acute or chronic modulation of gap junctions. Moreover, the development of connexin- or organ specific gap junction modulator drugs is a challenging task for pharmacologists.

Note: 2066 papers have been published so far concerning somehow the pharmacology of gap junctions and have been checked for this article. The present review here is focussed (not limited) on the developments for the last 5 years. Due to space limitations we were not able to include the complete list of all references regarding this area and tried to limit this list to those which are directly concerned with pharmacology. Several important articles may have not been included due to the space limitations and we ask for the understanding of the reader.

Acknowledgement

This study was supported by a DFG-grant given to SD.

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