Toward the discovery of digitalis derivatives with inotropic selectivity

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Increased incidence and prevalence of congestive heart failure make the discovery of novel positive inotropes (i.e. agents that increase the peak force of contraction of the heart muscle) a priority. Cardiac glycosides currently in use are inadequate for effective treatment or even for the minimization of the associated discomfort and disability. These drugs also frequently cause dangerous toxic effects. Substantial research within the pharmaceutical industry has failed to identify a nonsteroidal 'digitalis replacement'. Hence, every effort should be made to create new positive inotropes. This review departs dramatically from the current thinking on the problem and reveals a new approach to the discovery of novel cardiotonic drugs with inotropic selectivity.

In the 1980s, studies on inotropic therapy for heart failure focused on drugs that inhibit cardiac phosphodiesterase activity, because such drugs can increase myocardial levels of cyclic AMP (cAMP) and enhance the contractility of the failing ventricle. Early optimism regarding these drugs was based on their favourable and apparently sustained haemodynamic effects and on early reports of dramatic clinical improvement in patients with advanced heart failure[1,2].

Paradise postponed

In 1989, milrinone, a bipyridine derivative and one of the most attractive of the phosphodiesterase inhibitors, was studied according to a protocol designed to show that it is preferable to the digitalis compound digoxin as inotropic therapy for chronic heart failure. In this first well-controlled, randomized trial of long-term administration[3], the multicentre trial group surprisingly found that digoxin improved left-ventricular function and exercise tolerance, and reduced the need for other therapy for worsening heart failure. Milrinone, on the other hand, was associated with more side-effects, less evidence of improved left-ventricular function and of prevention of worsening of heart failure, and with a higher incidence of ventricular arrhythmias.

Remarkably, in cardiac tissue from heart failure patients, cAMP-dependent agents are relatively ineffective, so that such positive inotropic drugs lose their effectiveness just when they are needed most – in severe heart failure[4].

A multicentre, double-blind, randomized, placebo-controlled trial of oral enoximone, a newer phosphodiesterase inhibitor[5], did not demonstrate an improvement in exercise capacity or symptoms with enoximone therapy compared with placebo in patients with congestive heart failure receiving digoxin and diuretics. Neither did it provide evidence that enoximone is beneficial in long-term therapy of chronic heart failure.

In fact, the result of vast effort to develop a chronically and orally administrable drug to replace or even supplement digitalis has generally been disappointing. As stated in 1992 (Ref. 6), the initial excitement about the phosphodiesterase

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III inhibitors (for example, amrinone, milrinone and enoximone) was tempered by the results of large, well-designed trials indicating variable effectiveness and a prominent adverse-effect profile. In addition, the finding that repeated oral administration of milrinone can increase mortality in heart failure had a devastating effect on the further development of this class of drugs. In consequence, there has been little decline in the use of digoxin, indicating that the newer treatments for congestive heart failure have not replaced the widespread use of digitalis[7].

The most important predictor of response to therapeutic intervention in heart failure appears to be the effect of the agent on neurohormonal systems[8]. In general, drugs that reduce the activity of the sympathetic nervous system, as do the digitalis compounds, reduce the risk of worsening heart failure. Conversely, drugs that stimulate the sympathetic nervous system, as do phosphodiesterase inhibitors, increase cardiovascular morbidity and mortality[8]. As reviewed in 1994 (Ref. 9), the excess mortality associated with milrinone in a large survival study helped to close the door on this approach to the long-term therapy of heart failure – paradise postponed[10].

Need for selective inotropes

Had the therapeutic value of digitalis compounds not been popularized by Withering more than 200 years ago, modern regulatory agencies would probably have judged this class of drugs too toxic to be approved for clinical use. Since then, countless patients suffering from congestive heart failure have been helped by digitalis preparations; no doubt the demise of countless others was hastened by their application[11]. Although in the 1980s some investigators had raised doubts about the efficacy and safety of digitalis compounds, more recently several controlled trials convincingly showed that digoxin is an effective agent in the treatment of patients with chronic heart failure in normal sinus rhythm[12]. At least at present, a digitalis compound is the agent of choice if a positive inotropic drug is thought to be necessary in chronic heart failure[13]. In end-stage heart failure specifically, treatment with cardiac glycosides may still be effective, because they are cAMP-independent positive inotropic agents[14].

To manage chronic heart failure, efforts need to be made to generate new compounds that will not only beneficially affect the haemodynamic and functional impairment of patients with heart failure, but also hopefully contribute to the attainment of an achievable goal – prevention of the clinical manifestation of congestive heart failure[15]. In the light of accumulated evidence that the therapeutic ratios of various cardioactive steroids are fundamentally similar, it must be asked whether naturally occurring or synthetic compounds can be found with better separation between therapeutic and toxic effects. Even if myocardial toxicity resulting in arrhythmias is directly linked to the desired inotropic effect, advantage could be taken (in principle) of the fact that neural mechanisms are important in the mediation of electrophysiologic and most unwanted extracardiac effects of digitalis compounds. The clear clinical importance of the issue should encourage investigators to continue to seek derivatives with an improved therapeutic ratio over the agents that are currently in use[16].

Fundamental changes of conceptual bias

Since 1963 (Ref. 17) and 1964 (Ref. 18) most researchers have thought that both the inotropic and toxic digitalis effects result from moderate or stronger inhibition of Na^+/K^+-transporting ATPase (Na^+/K^+-ATPase) of the cardiac muscle plasma membrane[16]. This apparently intrinsic connection appears to account for the medical experience that the two actions cannot easily be separated by modification of the dosage regimen. The Na^+/K^+ pump lag hypothesis[18,19] accounts for both the therapeutic and toxic effects of digitalis by a single mechanism. If this is the only factor contributing to the therapeutic action of cardiac glycosides, then it is impossible to reduce their toxicity by chemical modification[20].

However, since 1972, various pharmacological observations, reviewed by Pastelin and Mendez[21], Okita[22], and Godfraind[23], have suppported the alternative view that Na^+/K^+-ATPase is not the unique cardiovascular digitalis receptor, but that two separate receptors are involved in mediating the inotropic and toxic digitalis effects. Okita[22] concluded that the receptor site associated with the positive inotropic digitalis action has weak binding affinities with rapid rates of both association and dissociation, while the receptor site associated with inhibition of Na^+/K^+-ATPase and with certain cardiotoxic digitalis effects has strong binding affinities with slow rates of association and a very slow rate of dissociation for the digitalis compounds. Moreover, Godfraind[23] stated that the structure–activity relationships are different for the interaction with high and low affinity sites. This observation suggested to him the possibility of designing a much safer drug devoid of inhibitory action on the Na^+/K^+ pump. Contrary to Okita[22], Godfraind[23] concluded that the inotropic mechanism, which is unrelated to changes in Na^+ and K^+ gradients, results from the interaction

of digitalis with high affinity sites. In a review published in 1985, entitled *Multiplicity of cardiac glycoside receptors in the heart*, Erdmann and coworkers[24] state that there is a serious discrepancy between the medically effective cardiac glycoside levels in patients (nanomolar range) and the glycoside concentrations required to elicit the positive-inotropic effect on isolated human papillary muscles (micromolar range). They concluded that there is, at present, no logical explanation for the discrepancy, but if two types of digitalis receptors can be distinguished in human heart, then there is hope for the development of new, more specific and possibly safer drugs to be used in myocardial failure.

Discovery of isoforms of receptor Na^+/K^+-ATPase

The apparent incompatibility of the above two lines of reasoning (namely that Na^+/K^+-ATPase is the unique digitalis receptor v. Na^+/K^+-ATPase is only one of two receptors, the other's identity staying open) was recently resolved by the demonstration that Na^+/K^+-ATPase, although remaining the only receptor, occurs in both cardiac muscle and nervous tissues in three isoforms called α1, α2, and α3 (Refs 25–28). This appears to open a new avenue for the discovery of digitalis derivatives that could inhibit preferentially one or other of the isoenzymes and could thus limit toxic effects[29]. It was expected that knowledge of which isoform, or isoforms, is the therapeutic receptor in the heart would enable investigators to design isoform-specific agents that should inhibit those cardiac isoforms linked to positive inotropy, but not those linked to toxicity[30]. Unfortunately, as will emerge later, this is an oversimplification.

Location and functional role of Na^+/K^+-ATPase isoforms

The fact that the α-subunit genes of Na^+/K^+-ATPase are strongly conserved in evolution[31] suggests that each α isoform has a specific biological role. The functional significance of the various isoforms can tentatively be derived from their predominant location in cardiac muscle. The α1-isoform is diffusely found in the plasma membrane, whereas the α3-pump protein is localized to sites for impulse transmission, namely the heart conduction system and the junctional complex between cardiac myocytes[32]. The observation that the myocardium is less sensitive to ouabain than the Purkinje fibres[33] suggests correspondingly graded digitalis sensitivities of the α1- and α3-isoenzyme.

Remarkably, the Na^+/K^+-ATPase isoform located in the sympathetic nerve endings[34], which has superior digitalis affinity (cf. below), is primarily involved in both the inotropic and toxic digitalis actions. Its inhibition leads to extracellular norepinephrine accumulation[35], which elicits the primary, effect-amplifying cAMP cascade resulting in the well-known catecholamine inotropy[36]. In relation to the total, the share of inhibited pumps involved in triggering the norepinephrine accumulation is so small that no change of global Na^+/K^+ movements can be observed[37]. Higher digitalis concentrations, which increase in parallel the degree of pump inhibition and catecholamine release, produce myocardial toxicity[16]. Hence, inotropic agents that improve haemodynamics at the expense of an increased 'adrenergic receptor stimulation' may adversely affect prognosis[38].

In conclusion, the Na^+/K^+-ATPase isoform population residing in the adrenergic nerve endings serves only initially as an inotropy-linked receptor. However, with increased dosage it becomes a toxicity-related receptor with the percentage of digitalis-inhibited enzyme entities increasing simultaneously. A pathophysiologic hallmark of heart failure is excessive activation of the sympathetic nervous system[39]. Therefore, a desirable agent to treat heart failure is likely to be one that improves haemodynamics without further activation of the sympathetic system[38].

Dual mechanisms underlie both the inotropic and toxic digitalis actions

The evidence for high digitalis affinity of the early inotropy-linked and finally toxicity-linked isoenzyme is reinforced by the following data. The blood serum concentrations connected with the therapeutically beneficial or the toxic actions in patients with cardiac failure lie between 1.3–2.6 nM and 2.6–4.8 nM digoxin, respectively[40]. Remarkably, the concentration/inhibition curves with Na^+/K^+-ATPase preparations from human cardiac muscle show only about 2–5% inhibition with 1–5 nM ouabain[41]. On the other hand, the concentrations producing positive inotropic effects on papillary muscle strips from human hearts lie between 3 and 1,000 nM ouabain[14]. The absence of toxic effects above the high concentration range indicates that the norepinephrine stores in the sympathetic nerve endings became exhausted during the laborious preparation of the muscle strips[42]. The above interrelations can be explained as follows:

- The therapeutically exploitable, narrow range of digitalis concentrations is fixed by the early intervention of the high-affinity, low-capacity Na^+/K^+-ATPase isoform of sympathetic nerve endings (for the sake of simplicity, named the 'toxicity-linked receptor').

- The medically attainable inotropic action of digitalis compounds does scarcely involve the low-affinity isoenzyme of cardiac muscle plasma membrane (for simplicity's sake named the 'inotropy-linked receptor') and hence does not reach its full potential[43].

The Na^+/K^+-ATPase isoform of low digitalis affinity, located in the plasma membrane of the cardiac muscle cell, regulates the chemical activity of intracellular Na^+ and in this way, via transmembrane Na^+/Ca^{2+} exchange, the intracellular Ca^{2+} activity. Inhibition of that Na^+/K^+-ATPase isoform hence results in increased Ca^{2+} activity and in correspondingly graded inotropic response[44]. Toxic digitalis levels, causing excessive inhibition of the Na^+/K^+-pump, lead to arrhythmias and ventricular fibrillation related to decreases in the intracellular $[K^+]$ and increases in the extracellular $[K^+]$ (Ref. 19).

Emerging research strategy

Both the fact that digitalis compounds can exert sympathoexcitation and that progression of heart failure is related to excessive activation of the sympathetic nervous system suggest that the required agent to treat heart failure should show low affinity to the Na^+/K^+-ATPase isoform in sympathetic nerve endings as well as high affinity to the Na^+/K^+-ATPase isoform in plasma membrane of cardiac muscle. The search for such digitalis derivatives by using Na^+/K^+-ATPase preparations in a prescreening system[45–47] presupposes that the isoenzymes involved in the disparate actions can be identified. This identification will be approached here via interrelation of the digitalis concentrations that trigger the various actions.

Identification of the inotropy- and toxicity-linked isoforms of Na^+/K^+-ATPase

The three isoenzymes cannot be biochemically separated because of physicochemical similarity[48]. Their digitalis affinities are not predictable from the differences in the amino acid sequences of the digitalis-intercalating subunits[47]. However, several pieces of circumstantial evidence for the assignment of the differing digitalis affinities to the three isoenzymes are available.

The EC_{50} value for the positive-inotropic effect of ouabain on papillary muscle strips from human hearts is about 80 nM (Ref. 14). This concentration is close to the IC_{50} value of ouabain for human α1 Na^+/K^+-ATPase, which is about 100 nM (Ref. 49). This interrelation indicates that the α1-isoenzyme is the inotropy-linked digitalis receptor. If one applies

Table 1. Different properties of the Na^+/K^+-ATPase preparations with regard to the kinetics of their inhibition by the clinically used cardiac glycosides (1–3) and the pharmacologically favoured derivatives (4–7)[a]

	Name of inhibitor	Enzyme[b] origin	IC_{50} (μM)	k_{on} (μM^{-1} min^{-1})	k_{off} (min^{-1})	τ/2 (min)
1	Ouabain	HH	0.027	1.2	0.033	21
		HK	0.035	0.3	0.011	63
2	Digoxin	HH	0.031	1.6	0.049	14
		HK	0.056	0.4	0.020	35
3	Digitoxin	HH	0.008	2.8	0.022	32
		HK	0.021	0.9	0.019	36
4	Actodigin	HH	2.4	0.18	0.43	1.6
		HK	0.92	0.94	0.86	0.8
5	(23*R*)-23-Methyl-actodigin	HH	0.31	1.4	0.44	1.6
		HK	0.15	3.4	0.50	1.4
6	(23*S*)-23-Methyl-actodigin	HH	1.9	0.24	0.45	1.5
		HK	0.67	0.47	0.32	2.2
7	3β-*O*-(β-D-glucopyranosyl)-3α-methyl-digitoxigenin	HH	0.81	1.3	1.0	0.7
		HK	0.54	0.97	0.52	1.3

[a]The Na^+/K^+-ATPases from human heart and kidney were prepared according to well-established procedures[60,61]. The enzymatic activity and the inhibitory potency of the digitalis compounds were determined as detailed elsewhere[62].
[b]HK, human kidney, this preparation essentially contains the α1-isoform; HH, human heart, contains an approximate 2:1 mixture of the α1- and α3-isoforms.

the wide gradation of digitalis affinities found with the three rat isoenzymes[50], the α3 Na^+/K^+-ATPase isoform is the high-affinity, toxicity-linked digitalis receptor. The blood serum levels of digoxin connected with the toxic actions are between 2.6 and 4.8 nM (Ref. 40). In other words, the digitalis affinity of the human α3-isoenzyme is at least 20-fold higher than that of the α1-isoform. These assignments allow an approximate interpretation of the isoenzyme base for the differing IC_{50} values measured for ouabain with the Na^+/K^+-ATPase preparations from human cardiac muscle and kidney (Table 1).

Elaboration of prescreen tests

Two groups have investigated the amount of each isoenzyme that normal human cardiac ventricle muscle contains on average. They found values of 62.5% α1, 15% α2 and 22.5% α3 (Ref. 27) and 48% α1, 26% α2 and 27% α3 (Ref. 51), respectively. Failing human heart was found to contain 55% α1, 10% α2 and 35% α3 mRNA by Zahler *et al.*[27] and 18% α1, 30% α2 and 52% α3 mRNA by Shamraj *et al.*[51]. Apparently, the α3 isoform level is increased in the failing ventricle. On the other hand, human kidney enzyme preparation holds about 90 % α1-isoenzyme but only 5–10% α3-isoenzyme[26,30]. In a first approximation, the Na^+/K^+-ATPase preparation from cardiac muscle with its comparatively high percentage of α3-isoenzyme can be taken as candidate for the toxicity-linked receptor, whereas the enzyme preparation from kidney, because of its high percentage of α1-isoform, can stand for the inotropy-linked receptor.

Scrutiny of Table 1 reveals that with the medically used digitalis compounds (**1–3**), the IC_{50} values for the cardiac muscle enzyme are constantly lower than they are for the kidney enzyme, whereas with the pharmacologically favoured derivatives (**4–7**), the IC_{50} values for the kidney enzyme are considerably lower. Hence, as long as the pure isoenzymes are unavailable, the emerging interim strategy to predict differential, favourable or unfavourable, properties of digitalis derivatives is to compare the inhibitory susceptibility of the two enzyme preparations in prescreens as exemplified below.

Digitalis derivatives with superior therapeutic range

Between 1972 and 1981, various researchers confirmed by different methods that the partial-synthetic digitalis derivative actodigin (**4**, Figure 1) exhibits a faster onset of action as well as a greater and faster reversibility of its toxicity compared with the classic digitalis compounds ouabain (**1**), digoxin (**2**) or digitoxin (**3**), and shows a greater margin of safety (reviewed by Pastelin and Mendez[21], and Wiesner and Tsai[52]). The findings with (23*R*)-23-methyl-actodigin (**5**) and (23*S*)-23-methyl-actodigin (**6**) were consistent with those reported for actodigin. Compared with the cardiac glycosides (**1–3**), actodigin (**4**) caused significantly less electrophysiological toxicity in isolated Purkinje fibres[53]. In contrast to compounds **1–3**, actodigin (**4**) did not trigger automaticity in isolated papillary muscles, even at high concentrations[54]. These observations appear to favour the early suggestion of Mendez (reviewed by Pastelin and Mendez[21], and Wiesner

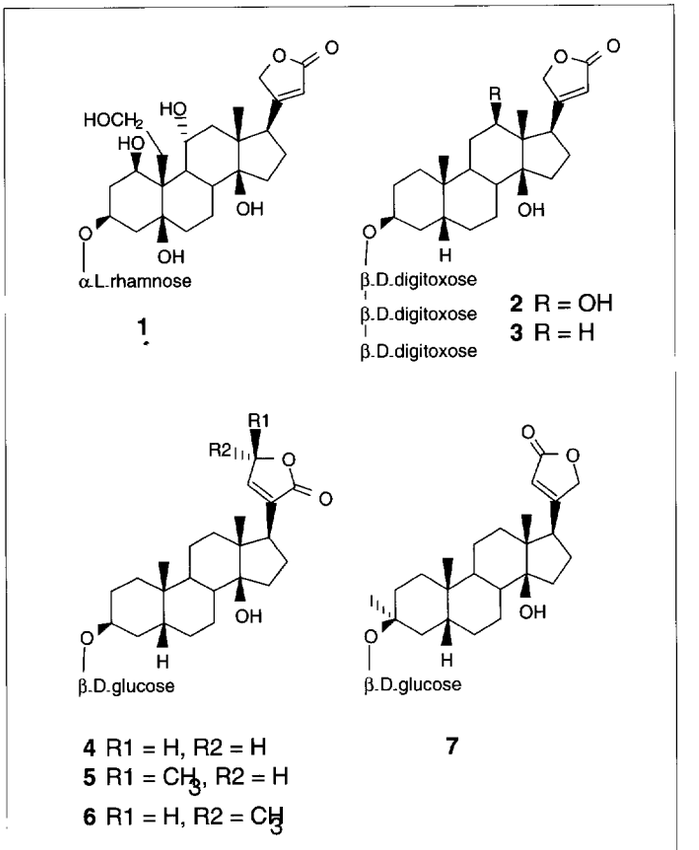

Figure 1. *Chemical structures of inhibitors of Na^+/K^+-ATPase generally used in the therapy of cardiac failure (**1–3**), or pharmacologically characterized (**4–7**) with respect to the ability to favourably differentiate between the toxicity- or inotropy-linked isoforms of Na^+/K^+-ATPase. **1**, ouabain; **2**, digoxin; **3**, digitoxin; **4**, 3β-(β-D-glucopyranosyl)-14β,24-dihydroxy-21, 23-bisnor-5β-chol-20(22)-ene-γ-lactone-20-carboxylic acid (actodigin); **5**, (23R)-23-methyl-actodigin; **6**, (23S)-23-methyl-actodigin; **7**, 3β-O-(β-D-glucopyranosyl)-3α-methyl-digitoxigenin.*

and Tsai[52]) that there are two separate receptors in cardiac muscle, one for the inotropic and another for the toxic actions, and that actodigin favourably distinguishes between the two. Possibly in the same vein, experiments on the action of 3β-*O*-(β-D-glucopyranosyl)-3α-methyl-digitoxigenin (**7**) in anaesthetized cats, in cat heart-lung preparations or in isolated guinea-pig left atria, yielded dose–response curves with flatter slopes and usually a higher inotropic maximum than was obtained with classical glycosides, thus revealing an improved therapeutic index (reviewed by Lüllmann and Mohr[55]).

Structure-based differentiation between the toxicity- and inotropy-linked receptor

The results of the present biochemical studies with Na^+/K^+-ATPase preparations from human heart and kidney (Table 1) appear to reveal the biochemical equivalents of the pharmacological findings with the clinically used digitalis compounds (**1–3**), the actodigins (**4–6**) and 3β-*O*-(β-D-glucopyranosyl)-3α-methyl-digitoxigenin (**7**). A comparison of the dissociation rate constants (k_{off}) shows that the digitalis derivatives have a faster reversibility of their action and hence much shorter half-life times (τ/2) of their inhibitory complexes. Taking the IC_{50} values, the most revealing distinction between the two types of agents is the inversion of the order of inhibitory susceptibility of the two Na^+/K^+-ATPase preparations. With the medically used digitalis compounds (**1–3**) the preparation from human heart, containing a high proportion of the toxicity-linked α3-isoenzyme, has the higher sensitivity, whereas with derivatives **4–7**, the preparation from human kidney, containing mainly the inotropy-linked α1-isoenzyme, shows the higher sensitivity.

Conclusion

This review presents an interim strategy to predict differential, favourable or unfavourable, properties of digitalis derivatives by comparing their inhibitory effect on Na^+/K^+-ATPase preparations from human kidney and cardiac muscle in prescreens. The two preparations contain different proportions of the α1- and α3-isoforms of Na^+/K^+-ATPase, which primarily serve as the inotropy- and toxicity-linked receptors, respectively. Through this methodology the biochemical equivalents of the pharmacological findings with the clinically used digitalis compounds and several digitalis derivatives distinguished by a greater margin of safety, have tentatively been identified. Nevertheless, the limited conclusiveness of the presently achievable methodology challenges prescreens of a wide variety of digitalis derivatives with the hitherto not available three pure isoforms of human Na^+/K^+-ATPase. In this way, digitalis derivatives with inotropic selectivity might be discovered. The ultimate question is whether or not they will provide novel therapeutic agents.

Epilogue

As pointed out recently by Chatterjee[56], any new therapy that could improve the prognosis of heart failure more than any therapy currently in use should be welcomed. Against this background, the potential benefits of the addition of a third-generation β-adrenergic antagonist (carvedilol) to the current therapy for clinical heart failure has been studied thoroughly[57–59]. The mechanism for the improved contractile response during chronic treatment with β-adrenergic antagonists, which in themselves exert a negative inotropic effect, has remained unresolved. Patients with more severe clinical heart failure are seen unlikely to benefit from β-blocker therapy, and there is increased risk of inducing worsening heart failure. In conclusion, carvedilol should be considered a therapeutic agent for prevention of progressive clinical heart failure rather than for treatment of refractory heart failure[56].

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