EFFECT OF ACTIVATION OF α- AND β-ADRENORECEPTORS AND OF MUSCARINIC ACETYLCHOLINE AND HISTAMINE H_1 RECEPTORS ON MECHANICAL TENSION IN FROG SUBCLAVIAN VEIN

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Attempts are currently being made to study the degree of heterogeneity of the different regions of the vascular system with respect to their pharmacological properties [4]. In the venous system of amphibians there are groups of neighboring regions (for example, the anterior vena cava, on the one hand, and the external jugular, innominate, and subclavian veins, on the other hand), which may have significantly different pharmacological properties, for they lie on different sides of the distal boundary of the pacemaker zone of the heart and differ sharply from one another in their functional characteristics [1].

The authors have begun an investigation to verify this hypothesis. The results of a study of the pharmacological properties of the subclavian vein are described below.

EXPERIMENTAL METHOD

Experiments were carried out on male winter frogs (*Rana temporaria*) aged 4-5 years. The pharmacological investigations were carried out on rings cut from the middle third of the left subclavian vein. The isometric tension was transformed by a strain gauge transducer based on tensometric resistors of the Yu-12B-77 type and recorded on a type KSP-4 potentiometer. The initial relative load was 10 mg/100 μ width of the ring. The composition of the physiological saline was described previously [1]. Experiments were carried out at 20°C. The contracting action of all substances except histamine was studied with respect to the cumulative response to increasing concentrations of the substance. For histamine, because of the unique character of its contracting action, separate responses were observed to each concentration of the series. The amplitude of the maximal contractile response was estimated by the index T_i/T_a, where T_i is the maximal height of response (maximal isometric tension − current passive load; to substance i; T_a the maximal height of the response to adrenalin (1×10^{-5} M). Values of the index T_i/T_a for all substances except histamine are given for the tonic component, but for histamine they are for the phasic component of the response. Relaxing effects were studied on the basis of the cumulative response. The value of maximal relaxation was assessed by the index $R_i/R(t)$, where $R_i = T(t_1) - T(t_2)$; $R(t) = |-T(t_1)|$; $T(t_1)$ represents the value of tension corresponding to the state of conditioning contracture (CC) at the beginning of observation of the relaxing effect; $T(t_2)$ is the value of tension corresponding to the state of CC established as a result of the maximal relaxing effect of the substance. CC was induced by 1×10^{-6} M adrenalin (CC_a) or by 6×10^{-2} M KCl (CC_{KCl}).

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TABLE 1. Substances Tested, Effective Concentrations (in pD_2 units), and Maximal Values of Response for Contraction and Relaxation (M ± m)

Substance	Contraction		Relaxation	
	pD_2	T_i/T_a	pD_2	$R_i/R(t)$
Adrenalin	7,1 ± 0,1 (n=8)	1	—	—
Noradrenalin	7,2 ± 0,2 (n=5)	1,0 ± 0,1 (n=3)	—	—
Isoproterenol	5,2 ± 0,1 (n=4)	0,5 ± 0,1 (n=6)	6,9 ± 0,1* (n=5)	0,6 ± 0,1* (n=5)
Dopamine	5,3 ± 0,1 (n=4)	0,5 ± 0,1 (n=3)	5,1 ± 0,05 (n=3)	0,6 ± 0,1 (n=3)
Histamine	6,6 ± 0,2 (n=6)	0,6 ± 0,1 (n=17)	3,5 ± 0,2 (n=3)	1,2 ± 0,2 (n=3)
Acetylcholine	Contracts in 40% of cases		6,7 ± 0,3 (n=6)	0,8 ± 0,1 (n=6)
Serotonin			4,6 ± 0,3 (n=3)	1,0 ± 0,1 (n=33)

Legend. $pD_2 = -\log(EC_{50})$. Values given in table, if not marked by asterisk, obtained when conditioning contracture was induced by adrenalin (1×10^{-6} M). Conditioning contracture was evoked by KCl (60 mM) in the presence of phentolamine (1×10^{-5} M). Number of experiments shown in parentheses.

Histochemical observations were made on the middle third of the left subclavian vein. The histochemical reaction for catecholamines was carried out with glyoxalic acid [3] and for acetylcholinesterase by the method in [5].

EXPERIMENTAL RESULTS

The action of adrenalin, noradrenalin, isoproterenol, dopamine, histamine, acetylcholine, and serotonin and modulation of their effects by appropriate antagonists were investigated.

Adrenalin, noradrenalin, isoproterenol, dopamine, and histamine were found to cause contraction in 100% of animals, acetylcholine did so in 40% (in 10 of 25 frogs), but serotonin had no contracting action. Isoproterenol, dopamine, histamine, acetylcholine, and serotonin lowered the tone of CC_a; isoproterenol, acetylcholine, and serotonin caused a fall in the tone of CC_{KCl}, but adrenalin, dopamine, and histamine had no such effect. The values of pD_2, T_i/T_a, and $R_i/R(t)$ for the contracting and relaxing effects are given in Table 1.

The α-adrenoblocker phentolamine (1×10^{-5} M) prevented, whereas the β-adrenoblocker propranolol (1×10^{-5} M) did not modify the contracting action of adrenalin, noradrenalin, and isoproterenol in concentrations exceeding EC_{50}. Phentolamine (1×10^{-5} M) prevented, whereas the dopamine receptor blocker haloperidol (2×10^{-6} M) did not modify the contracting action of dopamine (1×10^{-5} M). The histamine H_1 receptor blocker suprastin (1×10^{-6} M) suppressed, whereas the H_2-blocker compound IÉM-1044 (1×10^{-4} M) did not modify contraction induced by histamine (3×10^{-5} M). The latter likewise was unchanged by phentolamine (1×10^{-5} M).

Propanolol (1×10^{-5} M) reduced the value of $R_i/R(t)$ for isoproterenol to zero, but reduced it by only 25% under CC_{KCl} and CC_a conditions respectively. Phentolamine (1×10^{-5} M) did not block the relaxing action of isoproterenol under CC_{KCl} conditions. Haloperidol (2×10^{-6} M) did not affect the relaxing action of dopamine under CC_a conditions. Suprastin (1×10^{-5} M) and compound IÉM-1044 (3×10^{-4} M) did not modify the relaxing action of histamine under CC_a conditions. The muscarinic cholinolytic atropine (1×10^{-6} M) changed pD_2 for the relaxing effect of acetylcholine to 3.5 ± 0.7 (n = 3), whereas the nicotonic

cholinolytic tubocurarine (3×10^{-5} M) did not modify the relaxing action of acetylcholine. Blockers of serotonin receptors of M type (morphine, 5×10^{-5} M) and D type (LSD, 2×10^{-6} M) did not modify the relaxing action of serotonin under CC_a conditions (not tested under CC_{KCl} conditions).

In the histochemical tests no products of reactions with catecholamines and acetylcholinesterase were found in the wall of the subclavian vein, evidence of the absence of adrenergic endings (or other parts of neurons) and of endings containing acetylcholinesterase in them.

The high sensitivity of the preparation to adrenalin and noradrenalin to isoproterenol (as shown by relaxation to CC_{KCl}), histamine, and acetylcholine (Table 1), and also the results of experiments with blockers indicate the existence of α- and β-adrenergic, histamine H_1-, and muscarinic acetylcholine receptors in the wall of the subclavian vein of *R. temporaria*. Excitation of the α-adrenergic and histamine H_1-receptors increases, whereas excitation of β-adrenergic and muscarinic acetylcholine receptors reduces the tone of the vessel wall. The absence of nerve endings in the wall of the subclavian vein means that α- and β-adrenoreceptors are located on the muscle cell membrane. Histamine receptors evidently are similarly distributed. Muscarinic acetylcholine receptors responsible for relaxation of the arterial wall in warm-blooded animals are located on endothelial cells [2]. The suggestion that muscarinic acetylcholine receptors have a similar distribution in *R. temporaria* was not verified.

The low values of pD_2 and T_i/T_a (Table 1) and also the results of experiments with blockers indicate that the contracting action of isoproterenol and of dopamine is not effected through specific receptors for these substances. The low values of pD_2 and the results of experiments with blockers indicate that there is a nonspecific mechanism of realization of the relaxing effects of dopamine and histamine. This hypothesis is valid also for serotonin, at least so far as the possibility of its action through serotonin receptors of M and D types is concerned. Despite the nonspecific mechanism for realization of the relaxing action of histamine and serotonin, it is characterized by high values of $R_i/R(t)$.

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