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Pharmacological Activity of Some Neuro-Transmitters in the Isolated Thoracic Duct of Dogs

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Summary

This was an investigation of the effects of some drugs on the smooth musculature of the canine thoracic duct isolated in vitro. The alterations of tonic activity of the experimental preparation, induced by these drugs, reveal the existence of specific receptors. In particular, the contracting action of catecholamines suggests that the activation of receptors of the alpha-adrenergic type is involved in the modulation of this vessel's dynamics. On the other hand, the sensitivity of the preparation to the action of acetylcholine suggests the presence of cholinergic receptors as well.

Introduction

The anatomical structure of lymphatic vessels includes, in addition to an external adventitia and an internal endothelium, also a tunica media made of smooth muscle fiber cells arranged in several layers with a helicoid pattern of variable pitch (1, 2).

This musculature assists the drainage of lymph by providing a propulsive contractile activity, which was observed by several investigators. *Smith* (3) and *Webb* (4) described spontaneous rhythmic contractions in the lymph vessels of cats, mice, rats, and guinea pigs. *Mislin* (5), working with in-vitro preparations, showed that appropriate physical stimuli, such as heat and pressure delivered to the inside of mesenteric lymphatic vessels of guinea pigs, produced a response in terms of peristaltic activity; and the same author demonstrated the effects of a number of drugs on the contraction rhythm and muscular tonus of the same preparation (6, 7). In man, the existence of contractile activity was demonstrated in pelvic and inguinal lymph vessels, and in the thoracic duct (8). Opinions are at variance, instead, about the existence and type of nerve supply to the lymphatic vasculature. Some authors (9, 10) believe that the lymph vessels are within the jurisdiction of the autonomic nervous system; others (11) regard the contractility of lymph vessels as secondary to circulatory variations.

In view of obtaining further information about the physiological forces that regulate the propulsive activity of the thoracic duct, we studied the effects of such drugs as would best demonstrate regulation of this vessel by the autonomic nervous system.

Material and Method

Our experiments were carried out with in-vitro preparations of the thoracic duct of dogs, obtained by removing the vessel to a length of about 5 cm starting from its outlet in the subclavian vein. The fragment, stripped of connective tissue, was placed in a 20-ml isolated organ bath containing Krebs-Henseleit solution saturated with 5% CO_2 in O_2 and adjusted thermostatically at 37°C. The composition of the bath solution in millimoles per liter was as follows: NaCl 110; KCl 4.8; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0; glucose 1.1; EDTA 0.002. The segment of thoracic duct was attached to a U.Basile strain-gage microdynamometer, which subjected

the preparation to a tension of 300 mg and recorded tension variations on a revolving lampblack drum, with an amplification giving a 1-cm stroke of the writing arm for 40 mg of tension.

With this preparation we tested the effects of the following substances: acetylcholine chloride; epinephrine bitartrate; atropine sulfate; barium chloride; caffeine sodium benzoate; phentolamine methanesulfonate (Regitin, Ciba); physostigmine sulfate; isoprenaline sulfate (Aleudrin, Boehringer); nor-epinephrine bitartratè (Noradrec, Recordati); papaverine hydrochloride; and propranolol hydrochloride. Further details are given in the accompanying tables and figures.

Results

Table 1 shows the minimum active concentrations of each test substance on the isolated thoracic duct of dogs.

Table 1. Effects of some drugs on the isolated thoracic duct of dogs. The drugs were added to the bath and allowed to act for 3 minutes. Each drug was tested on 3 thoracic duct preparations obtained from different dogs. The table shows the concentrations of the drugs in the nutrient fluid.

Drugs	Minimum active concentrations	Effect
Nor-epinephrine	2.13×10^{-7}M	Contraction
Epinephrine	1.36×10^{-7}M	Contraction
Isoprenaline	4.73×10^{-5}M	Contraction
Acetylcholine	1.65×10^{-5}M	Contraction
Barium chloride	4.80×10^{-3}M	Contraction
Caffeine	3.00×10^{-5}M	Relaxation

Nor-epinephrine and epinephrine produce a dosage-dependent contraction (Fig. 1), which responds to the action of α-adrenergic blocking agents, and to a lesser degree to β-adrenergic blockers (Table 2). Isoprenaline elicits contracting effects at concentrations about 300 times higher than epinephrine or nor-epinephrine (Fig. 1); this action is likewise antagonized by equal concentrations of phentolamine or propranolol (Table 2). The concentration of phentolamine that produces a 50% reduction of contractions induced by catecholamines is between 1.06×10^{-6}M and 3.55×10^{-6}M; that of propranolol is between 3.38×10^{-4} and 1.01×10^{-3}M. Also acetylcholine exerts a dosage-dependent contracting effect, which is antagonized by atropine and potentiated by physostigmine (1.54×10^{-5}M).

In addition, the smooth musculature of the thoracic duct responds to direct stimulants such as barium chloride, and also to the relaxant effect of caffeine (Table 1). The contracting action of barium chloride is antagonized by papaverine at a concentration of 2.5×10^{-4}M.

Discussion

The in-vitro preparation of canine thoracic duct proved sensitive to a number of drugs known to act directly upon smooth muscle fiber cells or upon specific receptors.

The contracting action exerted by catecholamines reveals the presence of receptor structures of the α-adrenergic type. Isoprenaline does not modify the tonus of the prepara-

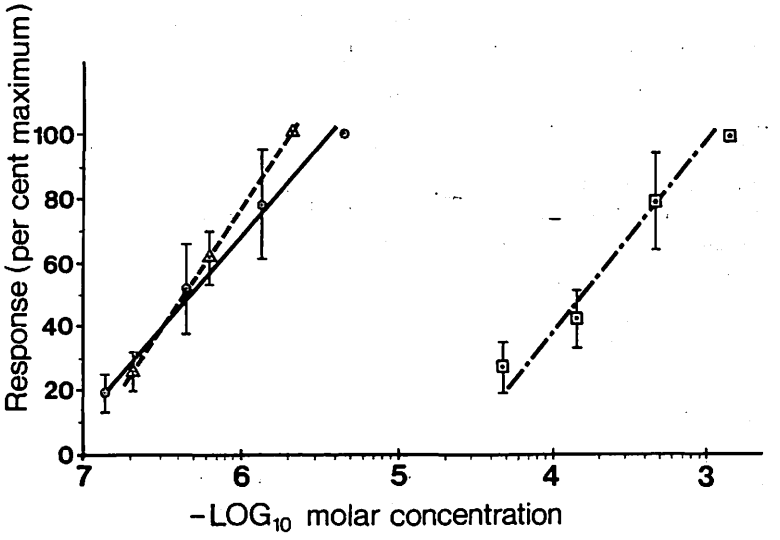

Fig. 1. Dose-effect curves for nor-epinephrine (Δ - - - - Δ), epinephrine (○ - - - - ○), and isoprenaline (□ - - - - □) on the isolated thoracic duct of dogs. The points represent the height of contraction of the preparation, expressed as per cent of the maximum height reached, induced by different concentrations of the drugs after 3 minutes' contact; each point is the mean of 3-4 responses. Vertical lines represent standard error.

Table 2. Effects of phentolamine and propranolol on contractions induced by nor-epinephrine, epinephrine, and isoprenaline on the isolated thoracic duct of dogs. The antagonist drugs were added to the bath 1 minute before the agonists.

Agonist		Antagonist		Per cent inhibition* ± s.e.
Nor-epinephrine	2.13×10^{-6}M	Phentolamine	1.06×10^{-6}M	30 ± 5.0
			3.55×10^{-6}M	73 ± 7.5
Epinephrine	4.55×10^{-6}M	Phentolamine	1.06×10^{-6}M	33 ± 7.5
			3.55×10^{-6}M	69 ± 4.0
Isoprenaline	1.42×10^{-3}M	Phentolamine	1.06×10^{-6}M	29 ± 3.0
			3.55×10^{-6}M	63 ± 2.9
Nor-epinephrine	2.13×10^{-6}M	Propranolol	3.38×10^{-4}M	48 ± 7.3
			1.01×10^{-3}M	87 ± 1.7
Epinephrine	4.55×10^{-6}M	Propranolol	3.38×10^{-4}M	33 ± 7.0
			1.01×10^{-3}M	85 ± 1.5
Isoprenaline	1.42×10^{-3}M	Propranolol	3.38×10^{-4}M	31 ± 5.2
			1.01×10^{-3}M	72 ± 2.9

*Mean of 4 experiments

tion at concentrations equimolecular to those of epinephrine and nor-epinephrine; and the contracting effects that appear with concentrations of isoprenaline 300 times higher than those of the two catecholamines are antagonized by phentolamine and seem attributable to activation of α-adrenergic receptors. The antagonistic action of propranolol on these three amines, being elicited only at very high concentrations (200 times

higher than those of phentolamine), must be regarded as nonspecific, probably a reflection of other pharmacological properties of this compound.

The contractions of the thoracic duct induced by acetylcholine, being blocked by atropine and potentiated by physostigmine, suggest the presence of a receptor of the muscarine type. Still, the high concentrations of acetylcholine needed to activate the thoracic duct musculature throw the role of the muscarinic receptor into relative unimportance in terms of overall thoracic duct motility. This view is corroborated by the fact that the catecholamines (nor-epinephrine and epinephrine) elicit contracting effects at concentrations much nearer to physiological values.

These preliminary observations reveal the complexity of phenomena involved in the regulation of lymph flow in the thoracic duct of dogs.

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