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THE PHARMACOLOGY OF A MOLLUSCAN SMOOTH MUSCLE

BY

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The effects of a number of pharmacologically active substances on contraction and on membrane polarization of the anterior byssal retractor muscle of *Mytilus edulis*, L., have been studied. Tetramethylammonium bromide, trimethyl(4-oxopentyl)ammonium chloride and nicotine, like acetylcholine, produced depolarization and sustained contraction. Nicotine, on repeated application, lost acetylcholine-like activity and effectively blocked acetylcholine. In order of decreasing potency, methanthelinium, tubocurarine, benzoquinonium, tetraethylammonium, atropine, pentamethonium, and decamethonium blocked acetylcholine action. These agents did not show initial acetylcholine-like action and did not relax sustained contractions. Adrenaline, noradrenaline, tyramine, dibenamine, phentolamine, and lysergic acid diethylamide relaxed sustained contractions without reducing initial depolarization and tension development in response to acetylcholine or electrical stimuli. Adrenaline and noradrenaline often caused depolarization and contraction when first applied, and displayed relaxing action on subsequent application.

By varying duration and frequency of stimulating electrical pulses, either a brief or a prolonged contraction may be evoked in the anterior byssal retractor muscles of *Mytilus edulis*, L. The prolonged contraction can be abruptly relaxed by repetitive electrical stimuli. These effects can be imitated by application of pharmacological agents, outstanding among these being acetylcholine and 5-hydroxytryptamine, which are naturally present in Mytilus muscle (Twarog, 1954) and ganglia (Welsh, personal communication). Acetylcholine produced depolarization and prolonged contraction. 5-Hydroxytryptamine promptly relaxed prolonged contractions. Acetylcholine and electrical stimuli, in the presence of 5-hydroxytryptamine, produced large, brief contractions and, often, rhythmic contractile activity.

Although the actions of acetylcholine, 5-hydroxytryptamine and adrenaline have been described in some detail (Twarog, 1954), the action of other substances on the anterior byssal retractor muscles has been only briefly reported (Twarog, 1952). The mechanisms of prolonged contraction and of relaxation are intrinsically interesting and the wide variety of agents affecting these phenomena is not generally known. It is the purpose of this note to make available some further, although incomplete, pharmacological results.

METHODS

In the study of acetylcholine-like, of acetylcholine-blocking and of relaxing agents, the muscle was mounted in a partitioned chamber to permit simultaneous recording of changes in demarcation potential and mechanical responses (Twarog, 1954).

In experiments comparing the relative acetylcholine-blocking efficiency of various agents, the muscles were mounted in upright chambers which were perfused with aerated sea water. Contractions were recorded on a moving drum by an isotonic lever weighted with 15 g. The height of contraction was measured before and after soaking for 2 min. in solutions of blocking agents, and % block calculated from the ratio of the magnitude of the response to acetylcholine after treatment to that before treatment.

RESULTS

Acetylcholine-like Agents.—Tetramethylammonium bromide, in concentrations above 5×10^{-6} M and trimethyl(4-oxopentyl)ammonium chloride (4-ketoamyltrimethylammonium chloride), an active stable analogue of acetylcholine (Welsh and Taub, 1950, 1951; Ing, 1949) in concentrations above 10^{-6} M, induced depolarization and a prolonged contraction. Characteristically, maximum depolarization was attained later than with acetylcholine. Depolarization followed the approximately exponential curve obtained after application of acetylcholine to a preparation treated with eserine.

Nicotine, below 10^{-4} M, potentiated the action of acetylcholine. Above 10^{-4} M, it evoked depolarization and prolonged contraction. However, tachyphylaxis was pronounced. With repeated applications of or continued soaking in nicotine, the

acetylcholine-like activity ceased. Loss of activity was associated with an acetylcholine-blocking action.

Acetylcholine-blocking Agents.—Nicotine, after acetylcholine-like activity had been lost, blocked acetylcholine very effectively. The blocking action could be reversed only with difficulty. Decamethonium, in concentrations lower than 5×10^{-4} M, potentiated acetylcholine action, suggesting some acetylcholine-like activity, weaker, however, than that of nicotine. Other substances have been studied which, in concentrations between 10^{-5} M and 5×10^{-4} M, blocked without displaying acetylcholine-like action. These are listed in Table I, in order of decreasing potency.

TABLE I

BLOCK OF THE ACTION OF 4×10^{-6} M ACETYLCHOLINE AT VARIOUS MOLARITIES OF SOME BLOCKING AGENT

An asterisk indicates potentiation of acetylcholine action.

Blocking Agent	% Block		
	10^{-5} M	10^{-4} M	5×10^{-4} M
Methanthelinium bromide ..	80	97	—
Tubocurarine	56	91	—
Benzoquinonium chloride ..	30	99	—
Tetraethylammonium	30	78	—
Atropine	20	98	—
Pentamethonium bromide ..	16	43	100
Decamethonium ,, ..	*	*	100

The acetylcholine-blocking drugs reduced depolarization by acetylcholine and tension development was also reduced to approximately the same degree as depolarization. The blocking action was reversible. The general form of contraction in response to acetylcholine was not altered, but tension was diminished and finally abolished entirely. These agents, applied during the phase of sustained tension after acetylcholine or electrical stimulation, did not relax the muscle.

Relaxing Agents.—Noradrenaline, in concentrations above 10^{-7} M, induced depolarization and prolonged contraction, summating with acetylcholine. When noradrenaline was not washed off, or was applied repeatedly in concentrations of 10^{-4} M or more, relaxation of tension ensued. No marked change in membrane polarization accompanied relaxation. With a maintained concentration of 5×10^{-4} M noradrenaline (in contrast to 10^{-6} M or 10^{-7} M 5-hydroxytryptamine), larger, but briefer, contractions followed acetylcholine or electrical stimulation.

The relaxing action of adrenaline, previously reported (Twarog, 1954), was more striking than that of noradrenaline. The concentrations at which these agents acted differed by about ten fold, adrenaline being the more potent.

Tyramine did not stimulate a resting muscle. In 10^{-4} M to 10^{-3} M tyramine, the contractile response to acetylcholine or electrical stimulation was increased while the sustained phase of contraction was abolished.

The anti-adrenaline agents dibenamine and phentolamine in concentrations of 10^{-5} M potentiated the contractile response to acetylcholine and electrical stimulation and relaxed prolonged tension (Fig. 1) as did higher concentrations of adrenaline, noradrenaline, and tyramine. The effect of these agents developed rather slowly and that of dibenamine appeared to be irreversible.

Certain lysergic acid derivatives also showed a relaxing action. This group included both anti-adrenaline agents and substances which affected or imitated the action of 5-hydroxytryptamine. Ergotamine and dihydroergotamine displayed no relaxing activity even in saturated solutions (about 10^{-4} M in sea water). Ergometrine, at 10^{-4} M or greater concentrations, effectively relaxed; it showed no excitatory action. Lysergic acid diethylamide (10^{-5} M) relaxed, again without producing initial depolarization or contraction.

DISCUSSION

Trimethyl(4-oxopentyl)ammonium ions were equal in potency to acetylcholine before but were about ten times less effective than acetylcholine applied to a muscle after treatment with eserine. Tetramethylammonium ions were about five times less potent than trimethyl(4-oxopentyl)ammonium ions. The curves of depolarization and repolarization closely resembled those for acetylcholine after eserine. Neither tetramethylammonium nor trimethyl(4-oxopentyl)ammonium ion would be inactivated by cholinesterase. This supports a previous conclusion that both potency and rate of depolarization and repolarization with acetylcholine are limited by cholinesterase activity in the fresh muscle.

The relative potencies of acetylcholine-blocking agents indicated that the anterior byssus retractor could not be classified as nicotinic or muscarinic, but was susceptible to agents blocking acetylcholine-like action at the neuromuscular junction and at autonomic ganglia as well as on smooth muscle of vertebrates. It resembled in some respects the heart of *Venus mercenaria* (Welsh and Taub, 1953; Luduena and Brown, 1952) except that benzoquinonium was not so outstandingly active. Significantly, these agents reduced the initial action of applied acetylcholine but, unlike the relaxing agents, did not affect tension maintenance. This could indicate that tension is maintained through a

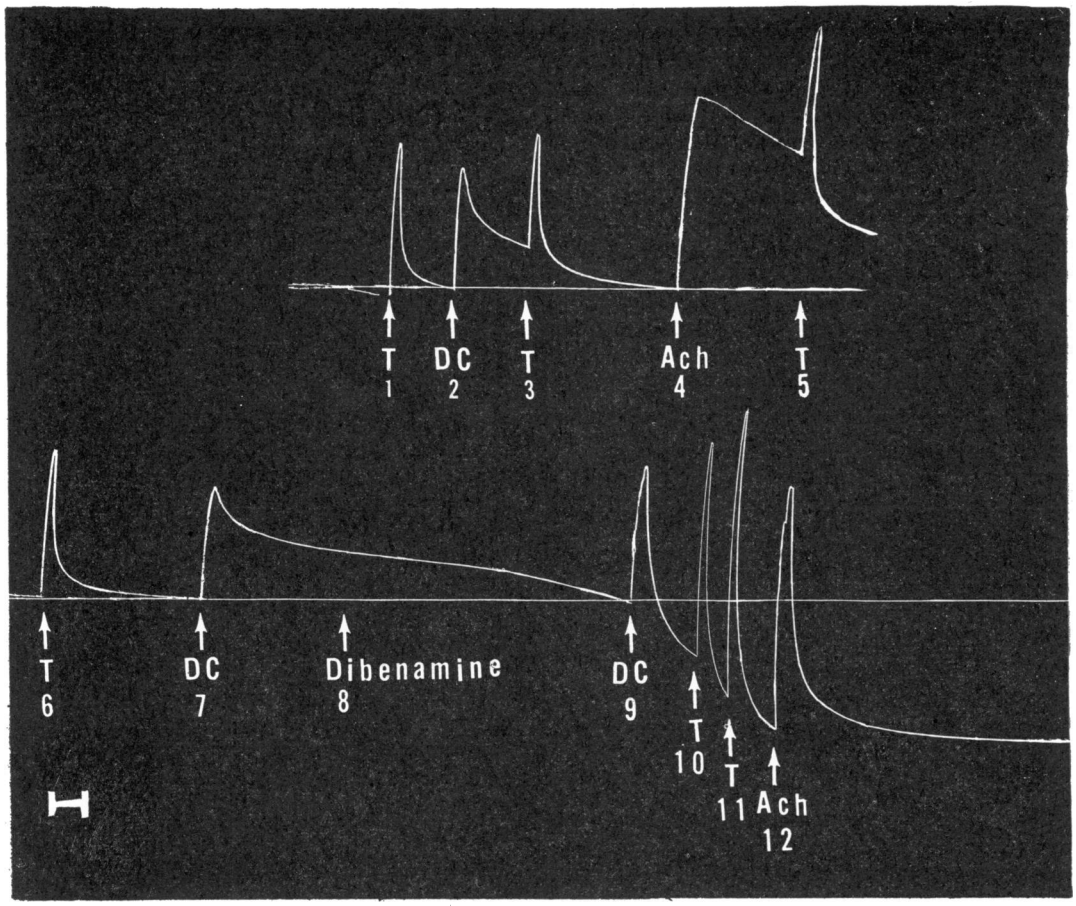

FIG. 1.—The effects of dibenamine on the responses of the anterior byssal retractor muscle of *Mytilus* to repeated, brief electrical pulses (T), single, long pulses (DC) and to acetylcholine (Ach). (1) 15 v. anodal, 20 msec., 10/sec. for 5 sec. (2) 3 v. cathodal, 5 sec. (3) 20 v. anodal, 20 msec., 10/sec. for 2.5 sec. (4) Ach 5×10^{-6} M for 10 sec. (5) 20 v. anodal, 20 msec., 10/sec. for 2.5 sec. (6) 5 v. anodal, 20 msec., 10/sec. for 3.5 sec. (7) 3 v. cathodal, 10 sec. (8) Dibenamine 10^{-4} M. (9) 3 v. cathodal, 10 sec. (10) 15 v. cathodal, 20 msec., 10/sec. for 2.5 sec. (11) 15 v. anodal, 20 msec., 10/sec. for 2.5 sec. (12) Ach 5×10^{-6} M for 15 sec. Time, 1 min.

non-cholinergic mechanism or is perhaps maintained without continued excitation (Twarog 1958; Johnson and Philpott, 1959). Another interpretation is that acetylcholine released at an intramuscular site might not be as susceptible to block as applied acetylcholine. Evidence against this is the observation that methanthelinium and benzoquinonium block neural excitation of the byssal retractor (Twarog, unpublished observation). However, this blocking action required concentrations five to ten times greater than those necessary for full block of applied acetylcholine.

Relaxation of pre-existing tension without block of tension development has been observed with 5-hydroxytryptamine and related agents in the smooth muscle of the gastropod radular apparatus (Hill, 1958; Fänge and Mattisson, 1958) as well as in the dorsal muscle of the leech (Poloni, 1955). Adrenaline relaxes the gastropod radula muscle (Hill, 1958).

Hoyle and Lowy (1956) observed relaxation of the byssus retractor with lysergic acid diethylamide, and this observation was confirmed. Here, as in the Venus heart (Welsh and McCoy, 1957) and in many vertebrate smooth muscles, lysergic acid diethylamide mimicked rather than blocked 5-hydroxytryptamine.

Of the ergot derivatives, ergotamine and dihydroergotamine, the most active anti-adrenaline agents, did not display a relaxing action, while ergometrine, a very poor anti-adrenaline agent and the most active uterine-stimulating agent, relaxed. However,

dibenamine and phentolamine relaxed very effectively.

The reversal of adrenaline and noradrenaline actions with repeated dosage at moderately high concentrations suggests a mechanism of block by the building-up of a local excess (Burn and Robinson, 1951). Relaxation might then be due to such a block. To some extent the action of anti-adrenaline agents lends support, but the relative activity of the ergots weakens the blocking hypothesis. Further, the connexion between the high-concentration effect of adrenergic agents and the actions of 5-hydroxytryptamine remains obscure. The phenomenon merits further attention, particularly in view of the interest which attaches to the distribution of noradrenaline, adrenaline, and 5-hydroxytryptamine in vertebrate nervous centres and the action of agents such as reserpine in relation to the release of catechol amines and of 5-hydroxytryptamine from storage sites in nervous tissue.

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