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ACETYLCHOLINE ANTAGONISTS: A COMPARISON OF THEIR ACTION IN DIFFERENT TISSUES

BY

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The work of Dawes (1946) on quinidine substitutes brought out points of resemblance which these substances possess apart from their action in cardiac tissue. Quinidine substitutes were found to include some local anaesthetics, some analgesic substances and some spasmolytic substances. Dawes pointed out that the local anaesthetic procaine possessed as much as 80 per cent of the action of quinidine on the refractory period of the isolated auricle, and that in addition it acted like atropine in diminishing the effect of acetylcholine on the isolated intestine. He also showed that procaine diminished the action of acetylcholine on the rate and force of the heart beat. The analgesic pethidine was found to have 83 per cent of the action of quinidine on the auricles, while the spasmolytics papaverine, syntropan and trasentin-6H had 50, 130 and 63 per cent respectively of this action.

I have therefore taken a group of seven substances, four of which are local anaesthetics: procaine, cocaine, amethocaine and nupercaine, the others being quinidine, trasentin-6H and atropine, and have compared them for their activity as local anaesthetics, for their action in modifying the stimulant action of acetylcholine (*a*) on the frog rectus and (*b*) on rabbit intestine, for their action in modifying the depression which acetylcholine produces in the rate and force of the heart beat, and finally for their power to modify the constrictor action of acetylcholine in the blood vessels of the rabbit's ear. The purpose of the comparison was to see whether they all possessed the same action towards acetylcholine and how closely the relative potency of these substances in one respect resembled the relative potency in another.

Local anaesthetic potency

The local anaesthetic potency of each substance has been tested, using the method described by Bülbring and Wajda (1945) of intracutaneous injection in guinea-pigs.

The relative local anaesthetic potency of these substances (taking 1 as the value of procaine) is shown in Table I.

TABLE I

LOCAL ANAESTHETIC ACTIVITY

Substance	Concentration producing the same effect g./100 ml.	Potency
Procaine ..	0.46	1.0
Cocaine ..	0.062	7.4
Amethocaine ..	0.060	8.0
Nupercaine ..	0.047	10.0
Quinidine ..	0.30	1.5
Trasentin-6H ..	0.46	1.0
Atropine ..	0.92	0.5

There is nothing surprising in the values obtained for cocaine, amethocaine and nupercaine. Quinidine might be expected, like quinine, to have a local anaesthetic action, but not be so strong as to exceed procaine in potency, as it does. It is surprising that trasentin-6H is equal to procaine, and that atropine has as much as 50 per cent of the action of procaine; both are stronger local anaesthetics than was expected.

A comparison of these substances in modifying the stimulant action of acetylcholine on the frog rectus

The isolated frog rectus muscle was suspended in a bath containing 7 ml. of frog-Ringer solution. The fluid was replaced by Ringer solution containing 10^{-7} acetylcholine every 5 min. and the stimulant effect of this substance on the muscle was recorded for 90 sec.; after washing out with normal Ringer the muscle relaxed to its previous extent. The response to this concentration of acetylcholine was observed at least three times at the beginning of each experiment and found to be the same. Then, 90 sec. before the next addition of acetylcholine, the fluid was changed to one containing a solution of the substance to be tested;

its own effect upon the muscle was recorded for 90 sec.; the action of this substance in modifying the stimulant action of acetylcholine was then determined. It was found that all seven compounds diminished the action of acetylcholine in a suitable concentration. In Fig. 1 (*a*) is shown the action of amethocaine added to the bath in a concentration of 0.5×10^{-4}. The record shows that the acetylcholine effect was greatly diminished for more than 20 min. When atropine and trasentin-6H were studied, an additional effect was observed, namely, that in a low concentration both these substances increased the action of acetylcholine. This is shown in Fig. 1 (*b*) for trasentin-6H in a concentration of 10^{-7}. Depression of the acetylcholine effect was obtained with trasentin-6H at 10^{-5}. The corresponding figures for atropine were, for augmentation 10^{-8} and for depression 6.6×10^{-5}. The results of comparing the different substances on the same preparation are given in Table II, which shows that the potency of the local anaesthetics on the rectus in relation to one another is in the same order as their local anaesthetic potency. Quinidine and atropine are also of similar relative potency to procaine, but trasentin-6H is much more potent on the frog rectus than as a local anaesthetic.

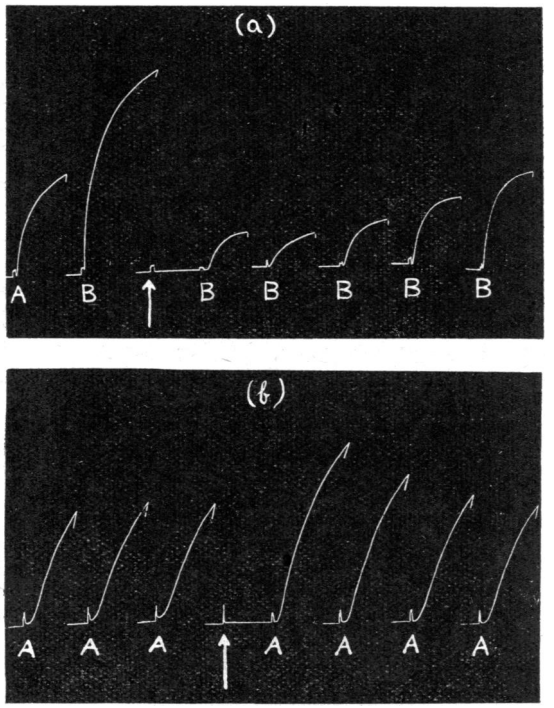

FIG. 1.—Frog's rectus muscle preparation.

(a) A, stimulant effect of acetylcholine added to the bath in a concentration of 5×10^{-8}. B, the effect when the acetylcholine added to the bath was in a concentration of 10^{-7}. At the arrow, amethocaine in a concentration of 0.5×10^{-4} was added to the bath. The record shows that the acetylcholine effect was greatly diminished.

(b) A, stimulant effect of acetylcholine added to the bath in a concentration of 10^{-7}. At the arrow trasentin-6H was added in a concentration of 10^{-7}. The acetylcholine effect was increased.

A comparison of these substances in modifying the stimulant action of acetylcholine on the rabbit intestine

The isolated rabbit's duodenum suspended in oxygenated Ringer at 34°C. was used. The spontaneous movements were recorded. To the bath of 75 ml. Ringer usually 4-10 μg. acetylcholine was added. The effect was observed several times in the same piece of intestine. When regular responses to the action of the same concentration of acetylcholine were obtained, the substance to be studied was added to the bath about 1.5 min. before the addition of acetylcholine; the action of the substance on the intestine and its effect on the response to acetylcholine were recorded. All seven substances diminished the stimulant action of acetylcholine on the rabbit intestine; there was no evidence of any increase of the stimulant action of acetylcholine when different concentrations of these substances were used. As shown in Table II it was found that cocaine and amethocaine had the same activity as procaine; nupercaine was 4 times, quinidine 6 times, trasentin-6H 1,500 times and atropine 3,000 times as active as procaine. Fig. 2 shows a comparison of the inhibitory effect of quinidine and atropine on the response of the intestine to acetylcholine.

A comparison of these substances in modifying the depression which acetylcholine produces in the rate and force of the heart beat

The isolated rabbit auricles were suspended in oxygenated Ringer at 29°C. and the spontaneous beat was recorded. Every 10 min. 0.4 ml. of an acetylcholine solution, containing 100 μg. per ml., was added to the 75 ml. Ringer; its depressing effect on the rate and force of the auricular beat was recorded. After the effect had been obtained the fluid in the bath was changed to allow the auricles to recover. It was found that while procaine, quinidine, trasentin-6H and atropine diminished the depression produced by the acetylcholine (Fig. 3a), cocaine had no effect (being

TABLE II

RELATIVE POTENCY IN TERMS OF PROCAINE

Substance	Inhibitory effect on the action of the acetylcholine							
	Frog rectus		Intestine		Auricle		Rabbit ear	
	Concentration	Potency	Concentration	Potency	Concentration	Potency	Dose μg.	Potency
Procaine	10^{-4}	1.0	2×10^{-5}	1.0	5×10^{-6}	1.0	100	1.0
Cocaine	3.3×10^{-5}	3.0	2×10^{-5}	1.0	5×10^{-6} 1×10^{-4}	nil	40	2.5
Amethocaine ..	1.1×10^{-5}	9.0	2×10^{-5}	1.0	5×10^{-6}	opposite effect	100	1.0
Nupercaine ..	9×10^{-6}	11.0	5×10^{-6}	4.0	5×10^{-6}	opposite effect	100	1.0
Quinidine ..	5×10^{-5}	2.0	3.3×10^{-6}	6.0	5×10^{-5}	0.1	400	0.4
Trasentin-6H ..	10^{-5}	10.0	1.33×10^{-8}	1,500.0	1.25×10^{-7}	40.0	10	10.0
Atropine ..	6.6×10^{-5}	1.5	6.7×10^{-9}	3,000.0	1.25×10^{-8}	400.0	2	50.0

tested in amounts from 0.5 mg. to 8 mg. in the bath of 75 ml.). Nupercaine and amethocaine (0.5 mg. each) actually increased the action of acetylcholine (Fig. 3b); smaller amounts (from 20 to 200 μg.) had no effect and larger amounts (2 mg.) arrested the spontaneous beating of the auricles. To obtain the same effect with quinidine as with procaine it was necessary to use a quinidine solution 10 times stronger, while the same effect was obtained with a trasentin-6H solution 40 times weaker, and with an atropine solution 400 times weaker, than procaine. These results are shown in Table II.

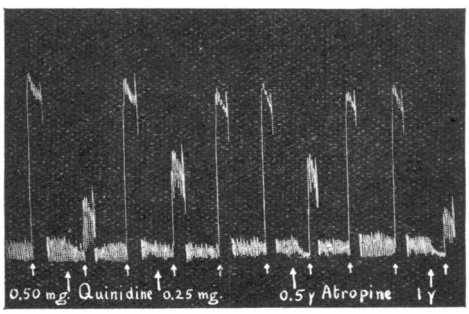

FIG. 2.—Isolated rabbit's duodenum. The record shows a comparison of the inhibitory effect of quinidine and atropine on the response of the intestine to acetylcholine. At the small arrows 10 μg. acetylcholine was added to the bath. The bath was washed out at each interruption of the record. At the first large arrow, 0.5 mg. quinidine sulphate was added to the bath; at the second, 0.25 mg. quinidine sulphate. At the third 0.5 μg. atropine sulphate and at the fourth 1.0 μg. atropine sulphate.

The effect of these substances on the constrictor action of acetylcholine in the blood vessels of the rabbit's ear

The carotid arteries of a rabbit under urethane anaesthesia were dissected and all their side

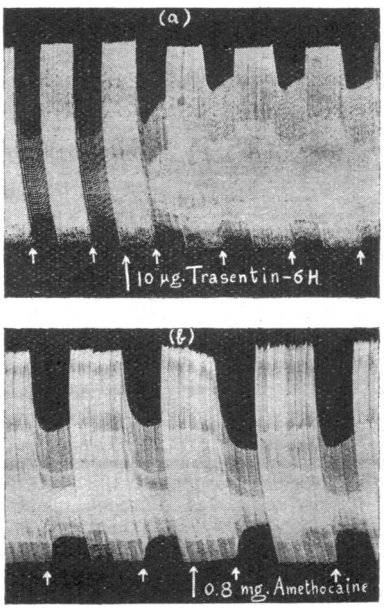

FIG. 3.—Isolated rabbit's auricles.
(a) Shows that the depression produced by 40 μg. acetylcholine (added to the bath at the small arrow) is greatly diminished after the addition of 10 μg. trasentin-6H. The bath was washed out 0.5 min. after each addition of acetylcholine.
(b) When 0.8 mg. amethocaine was added to the bath the depression produced by the acetylcholine was increased.

branches except the posterior auricular artery tied and severed as in the method of Gaddum and Kwiatkowski (1938). The jugular veins were then exposed and the anterior facial and posterior mandibular branches tied, leaving only the branch which drains the ear. Cannulae were inserted into both carotid arteries, the jugular veins were tied and incisions made in the veins proximal to these ligatures. The perfusion was then started. The animal was killed and the body detached from the head. Glass cannulae were introduced into the jugular veins. The outflow was determined by the Gaddum drop timer. The injections were made through a rubber cap covering the T-tube connected with the cannulae leading to the carotid artery. The volume of the injections was 0.2 ml. The constrictor effect of 100-200 μg. acetylcholine on the vessels was recorded on a smoked drum. It was found that these substances abolished the constrictor effect of acetylcholine. As shown in Table II, procaine, amethocaine and nupercaine had the same activity; quinidine was weaker; cocaine, trasentin-6H and atropine were stronger than procaine. These comparisons were made in six different preparations. Fig. 4 shows an example of this inhibitory effect when cocaine (100 μg.) was used.

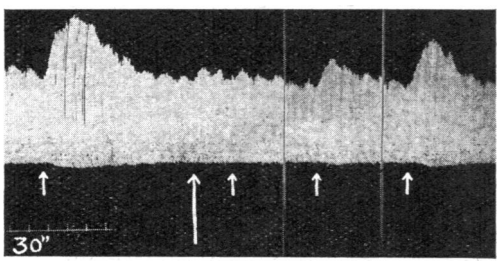

FIG. 4.—Rabbit's ear perfusion. Outflow recorded by Gaddum's drop-timer. The record shows the constrictor effect of 200 μg. acetylcholine on the vessels (injected at each small arrow); the injection of 100 μg. cocaine (at the longer arrow) abolished the constrictor effect of the acetylcholine.

DISCUSSION

The suggestion which initiated this work was that different agents, local anaesthetics, spasmolytics and quinidine all act fundamentally as antagonists, direct or indirect, of acetylcholine. It may be wondered why a local anaesthetic should be thought to have this effect. Harvey, Lilienthal, and Talbot (1941), however, record that when acetylcholine is injected intra-arterially it causes very severe pain, and Gray (1947) has shown that the injection of acetylcholine into an artery leading to a detached portion of the skin of the cat sets up an electrical disturbance in the nerve leaving the skin very similar to that produced by mechanical pressure on the skin. Further investigation may therefore indicate that a local anaesthetic is essentially a substance which opposes the action of acetylcholine at sensory nerve endings. No view is put forward concerning the mechanism of the antagonism; we do not know how, for example, procaine diminishes the stimulant action of acetylcholine on the frog rectus; it may be that procaine blocks the receptors to which acetylcholine must attach itself to produce stimulation; there are many other possibilities of which we are ignorant, and even the conception that the receptors are blocked is little more than a restatement of the observation made.

On the whole the investigation has shown that the different substances examined do possess an antagonistic action on all tissues affected by acetylcholine, whether skeletal muscle like the frog rectus, cardiac muscle like the rabbit auricle, or unstriated muscle like that of the intestine or of the blood vessels. This, however, does not in itself support the hypothesis mentioned above that local anaesthetics, spasmolytics and quinidine all act fundamentally as antagonists of acetylcholine, since it is probable that all these substances, in some concentration, would also antagonize histamine. Histamine is, however, not known to cause pain on intra-arterial injection, or contraction of skeletal muscle, or to have an action comparable with that of acetylcholine on cardiac muscle. Hence some significance can be attached to the antagonism of acetylcholine, though there would be much more in a demonstration that the relative potency of these substances in reducing the effect of a natural stimulus was the same as the relative potency in reducing the effect of acetylcholine in that tissue.

In different tissues the relative potencies of these substances are very different except that there is a similarity between local anaesthetic action and acetylcholine antagonism on the frog rectus. Atropine is 3,000 times more potent than procaine on the intestine, but only 50 times on the blood vessels. It is scarcely surprising that these quantitative differences are so great, for they explain the ordinary view that local anaesthetics, spasmolytics and quinidine-like compounds are unrelated in action, and have no common properties. If the relative potency of the different substances had been even approximately the same on the tissues examined, this would have been discovered long ago. The difference, however, does not disprove the hypothesis. It is known already that atropine and nicotine vary in their relative anti-acetyl-

choline action in skeletal and in plain muscle.

It is difficult to understand the behaviour of amethocaine and nupercaine on the rabbit auricle. In all the concentrations tested they increased the action of acetylcholine and did not depress it; cocaine, moreover, was without action, neither depressing nor augmenting. Nupercaine and cocaine are known to modify the action of the isolated heart of the cat, and also to augment the action of adrenaline on the isolated heart (Tripod, 1940), whereas procaine has no such effect. It is possible that the action of cocaine, amethocaine, and nupercaine in relation to acetylcholine is masked by another effect; the behaviour of these three substances on the auricle may not therefore disprove the rule.

Summary

1. An examination has been made of the local anaesthetics procaine, cocaine, amethocaine, and nupercaine, the spasmolytics trasentin-6H and atropine, and of quinidine.

2. These substances have been compared for their local anaesthetic action, and for their power to depress the action of acetylcholine on skeletal muscle (frog rectus), cardiac muscle (rabbit auricle), and unstriated muscle (rabbit intestine and blood vessels).

3. The local anaesthetic action of atropine is as much as half that of procaine, while that of trasentin-6H is equivalent to that of procaine.

4. All these substances depress the action of acetylcholine on the frog rectus, the rabbit intestine and the rabbit blood vessels. The relative potencies of the four local anaesthetics on the frog rectus are very similar to their relative local anaesthetic potencies.

5. Procaine, trasentin-6H, quinidine, and atropine depress the action of acetylcholine on the heart. Cocaine is without action, and amethocaine and nupercaine augment the action of acetylcholine in this tissue.

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