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THE PHARMACOLOGY OF BENZOYLCHOLINE

BY

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During a study of drugs possessing curare-like properties, Bovet and his co-workers (1949) noted that 50 mg./kg. of benzoylcholine caused head-drop in rabbits. It produced hypotension in dogs in doses of 0.1 mg./kg., and hypertension in higher doses. In addition, it caused contraction of the rabbit ileum and rectus abdominis muscle of the frog, and increased salivary secretion in dogs. Earlier, Carr and Bell (1947) found that benzoylcholine was much less active than acetylcholine in causing hypotension in dogs, and did not cause miosis in the rabbit's cornea. From these results it was concluded that benzoylcholine had strong nicotine-like properties and weak muscarine-like properties.

Because it contains a benzoic ester grouping, like many local anaesthetics, we first determined whether benzoylcholine possessed local anaesthetic properties. Secondly, we compared the actions of benzoylcholine and acetylcholine, and also the interactions between the two, with particular reference to the effect of benzoylcholine on heart muscle and the gut.

METHODS

The tests for local anaesthetic activity were carried out (*a*) on guinea-pig's skin (Bülbring and Wajda, 1945) and (*b*) on rabbit's cornea. For experiments on striated muscle, the isolated rectus abdominis of the frog (bath volume 2 ml.), the isolated phrenic nerve-diaphragm of the rat (bath volume 50 ml.), and the sciatic-gastrocnemius preparation of the cat under chloralose anaesthesia were used. Intravenous injections into week-old chicks (Buttle and Zaimis, 1949) were carried out in order to differentiate between true curare-like properties (flaccid paralysis) and decamethonium-like actions (spastic paralysis).

Pieces of rabbit and guinea-pig gut and guinea-pig uterus were suspended in an isolated organ bath (volume 15 ml.) containing Tyrode's solution at 37° C. For studying the peristaltic reflex, a large bath (volume 50 ml.) was used. Isolated tracheal chain preparations of the cat and the rabbit (Castillo and de Beer, 1947) were also used in a 15 ml. bath.

Cats anaesthetized with chloralose (60 mg./kg.) were used for studying effects on the arterial blood pressure recorded from the carotid artery. Rabbit and cat auricles were suspended in a bath of Locke's solution (15 ml.) at 30° C.; whole hearts were perfused by the Langendorff technique. Straub frog hearts were also used for studying the action of drugs on ventricular muscle.

The drugs used were benzoylcholine chloride (B.D.H.), acetylcholine chloride, choline chloride, eserine sulphate, *d*-tubocurarine chloride, hexamethonium bromide, nicotine acid tartrate, atropine sulphate, cocaine hydrochloride, and procaine hydrochloride. All were made up in solution in distilled water.

RESULTS

Local anaesthetic activity

Benzoylcholine (in 2 per cent solution) possesses no local anaesthetic property when tested by the standard procedure on the rabbit's cornea. On the shaved skin of the guinea-pig, however, slight activity is observed. The mean value of four determinations showed that benzoylcholine is an eighth as active as procaine by this test.

Action on striated muscle

On the isolated rectus abdominis of the frog, benzoylcholine produces contraction, but it is 5 to 20 times less active than acetylcholine. Its action is little affected by eserine, but tubocurarine (10^{-5}) and procaine (10^{-4}) block the response, just as they do that of acetylcholine. The presence of small doses of benzoylcholine in the bath does not affect the acetylcholine or benzoylcholine response.

When injected into week-old chicks under pentobarbitone or ether anaesthesia, benzoylcholine causes the spastic reaction typical of decamethonium. Benzoylcholine (1 mg./kg.) is more active than acetylcholine by this test and produces rigid extension of the limbs and retraction of the head. The muscle twitch of the cat

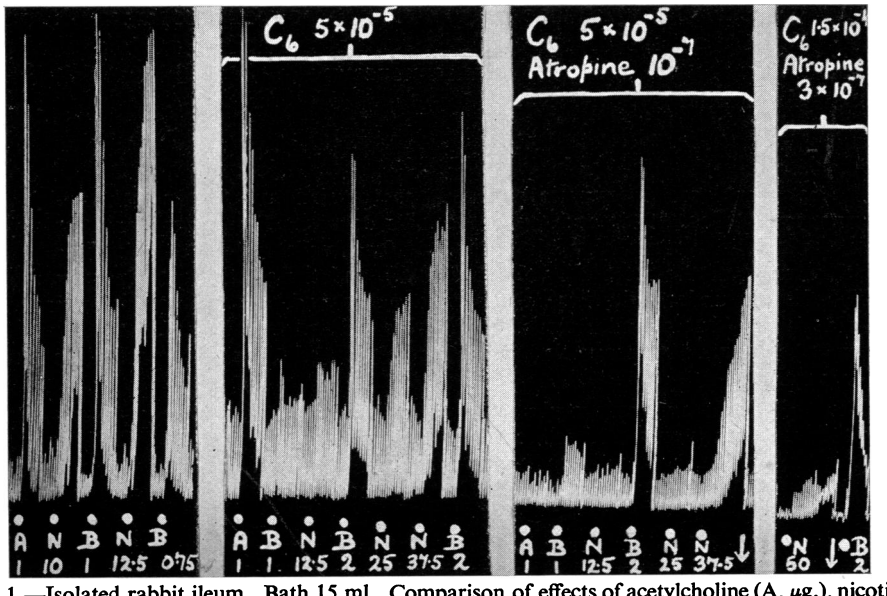

FIG. 1.—Isolated rabbit ileum. Bath 15 ml. Comparison of effects of acetylcholine (A, μg.), nicotine (N, μg.), and benzoylcholine (B, mg.). Hexamethonium (5×10^{-6}) blocks the benzoylcholine and nicotine responses. Larger doses of benzoylcholine are effective in hexamethonium and atropine when nicotine is ineffective. Time in min.

sciatic-gastrocnemius preparation, evoked by single maximal nerve volleys, is depressed by intra-arterially injected benzoylcholine (0.5–2 mg./kg.). This neuromuscular blocking action was also observed in the isolated phrenic nerve-diaphragm preparation of the rat (concentrations of 10^{-4}).

Action on smooth muscle

Isolated intestine.—On the rabbit ileum, benzoylcholine causes stimulation, though it is usually more than 1,000 times less active than acetylcholine (Fig. 1). Hexamethonium (5×10^{-5}) abolishes this stimulation just as it does that of nicotine. The initial stimulation, therefore, is caused by the nicotine-like property of benzoylcholine. Doubling the benzoylcholine dose after hexamethonium (5×10^{-5}) and atropine (10^{-7}) results in recovery of stimulation, whereas increasing the nicotine dose threefold only results in partial recovery (Fig. 1). In the presence of stronger concentrations of hexamethonium and atropine, the double dose of benzoylcholine is effective, whereas increasing the nicotine dose fourfold is ineffective (Fig. 1). Benzoylcholine, therefore, possesses both a hexamethonium-resistant and an atropine-resistant stimulant action.

A similar result was found in the rabbit duodenum. In the presence of hexamethonium an ineffective dose of benzoylcholine prevents both the acetylcholine and the nicotine responses without affecting the benzoylcholine response. Benzoylcholine, therefore, also possesses an atropine-like action.

On the isolated guinea-pig ileum, benzoylcholine is usually more than 4,000 times less active than acetylcholine in producing stimulation. Whereas in this tissue hexamethonium (5×10^{-5}) effectively abolishes the nicotine stimulation, it has little effect on that of benzoylcholine. In the presence of both hexamethonium (5×10^{-5})

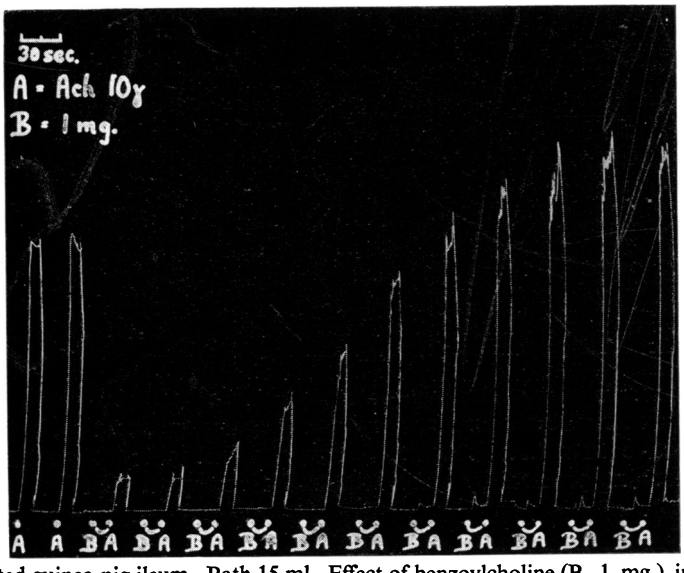

FIG. 2.—Isolated guinea-pig ileum. Bath 15 ml. Effect of benzoylcholine (B, 1 mg.) in the bath on the stimulation produced by acetylcholine (A, 10 μg.). Note that the acetylcholine response is first reduced and then recovers despite the presence ot benzoylcholine. Time in 30 sec.

and atropine (10^{-7}), twice the normal benzoylcholine dose is effective in stimulating the muscle, although increasing the nicotine dose tenfold is almost without effect. When mepyramine (10^{-9}) is added to the perfusing fluid, the action of benzoylcholine is little affected although the histamine response is blocked.

In another series of experiments on guinea-pig ileum, the action of benzoylcholine in blocking the acetylcholine response was further investigated. If the blocking action is repeated many times on the same preparation, an unusual effect is observed (Fig. 2). Slowly and steadily the acetylcholine response recovers despite the fact that effective blocking doses of benzoylcholine are still present in the bath. Very small doses of benzoylcholine ($10^{-7} - 5 \times 10^{-6}$) added before a standard acetylcholine dose do not potentiate its response, but potentiation is usually observed when benzoylcholine is washed out of the bath.

On the peristaltic reflex, large doses of benzoylcholine (2×10^{-4}) almost completely abolish the reflex in a manner similar to that shown by hexamethonium.

Isolated trachea of cat and rabbit.—In these preparations, small doses of benzoylcholine (10^{-5}) potentiate the acetylcholine response, medium doses (10^{-4}) block it, whilst larger doses *per se* (up to 10^{-3}) are without effect (Fig. 3).

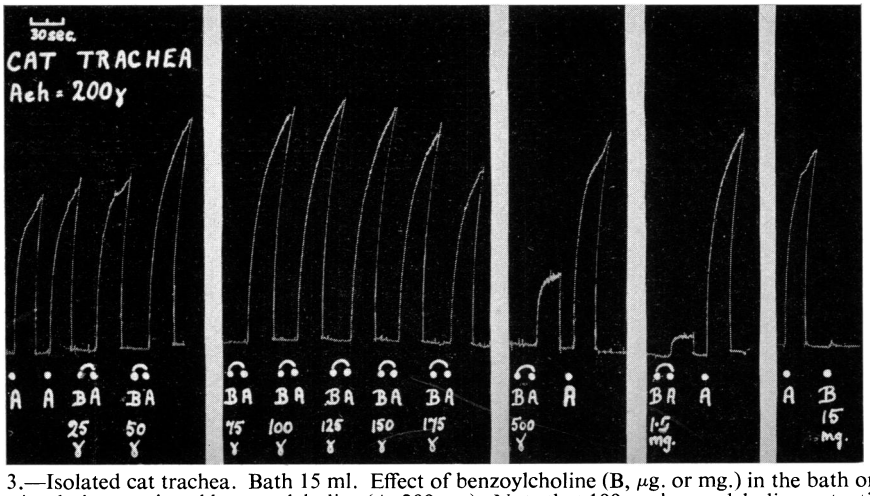

FIG. 3.—Isolated cat trachea. Bath 15 ml. Effect of benzoylcholine (B, μg. or mg.) in the bath on the stimulation produced by acetylcholine (A, 200 μg.). Note that 100 μg. benzoylcholine potentiates, and 500 μg. reduces, the acetylcholine response. Large doses of benzoylcholine (10^{-3}) are without effect in this preparation. Time in 30 sec.

Isolated uterus of guinea-pig.—Benzoylcholine ($2 \times 10^{-4} - 10^{-3}$) causes contraction of the uteri of both pregnant and non-pregnant guinea-pigs, but it is 200–400 times less active than acetylcholine. This muscle-stimulating action of benzoylcholine is not antagonized by concentrations of atropine effective against acetylcholine.

Action on blood pressure

In the freshly anaesthetized cat, an intravenous dose of benzoylcholine (0.1–0.2 mg./kg.) usually causes a transient fall in blood pressure. This fall is abolished by

atropine (1 mg./kg.) so that the effect is due to the muscarine-like property of benzoylcholine. Higher concentrations (0.8–1 mg./kg.) cause a rise in blood pressure. This rise is potentiated by intravenous doses of procaine (2 mg./kg.) or cocaine (1 mg./kg.), but is antagonized by hexamethonium (10 mg./kg.) or adrenalectomy. It represents the nicotine-like property of benzoylcholine. After hexamethonium or adrenalectomy, however, larger doses of benzoylcholine (4–5 mg./kg.) cause a rise in blood pressure, probably resulting from a direct stimulant effect on the muscle in the blood vessels. If the hind-limbs of the rabbit are perfused with Tyrode's solution containing atropine (10^{-6}), the injection of benzoylcholine (1–2 mg.) also causes vasoconstriction.

Action on heart muscle

Benzoylcholine stimulates the isolated rabbit or cat auricles. Small amounts produce a single delayed stimulation lasting about a minute; larger doses give a biphasic response, initially a rapid transient stimulation followed a few seconds later by a more prolonged stimulation. The initial rapid stimulant action can be blocked by doses of nicotine too small to have a stimulant action themselves, whereas very small doses of benzoylcholine given one minute before effective doses block both stimulant effects. Whereas hexamethonium (5×10^{-6}) prevents stimulation by nicotine, the benzoylcholine response is little affected either by hexamethonium or atropine (Fig. 4). In addition, benzoylcholine ($10^{-5} - 10^{-4}$) blocks the acetylcholine

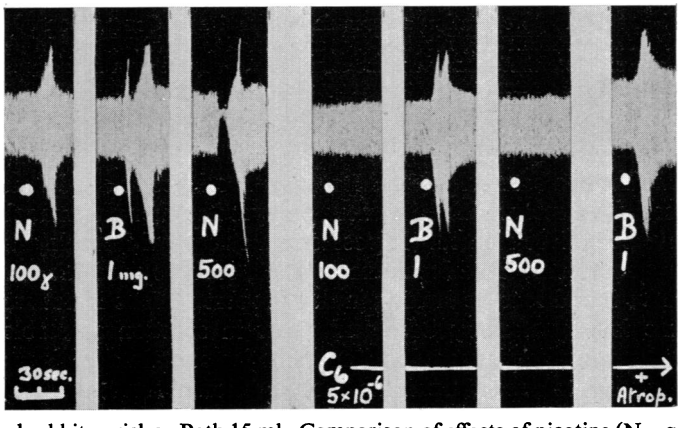

FIG. 4.—Isolated rabbit auricles. Bath 15 ml. Comparison of effects of nicotine (N, μg.) and benzoylcholine (B, mg.) before and after hexamethonium (5×10^{-6}). The stimulant action of benzoylcholine is not affected by hexamethonium or atropine (10^{-7}). Time in 30 sec.

response, and restarts the beat when excess acetylcholine is present. On the isolated perfused rabbit heart (Langendorff preparation) benzoylcholine (100–200 μg.) stimulated the beat, the effects being very similar to those seen in the auricle preparation.

Benzoylcholine increases the amplitude of the beat of the isolated frog heart (Straub preparation). This effect is not due to its nicotine-like property, since nicotine itself slightly inhibits the beat and benzoylcholine stimulates in the presence of nicotine (Fig. 5). A stimulant dose of benzoylcholine (50 μg.) prevents the acetyl-

choline or choline response and starts the ventricle when it has been stopped by acetylcholine or choline. Since this stimulant action of benzoylcholine is unaffected by atropine, it probably represents a direct action on cardiac muscle.

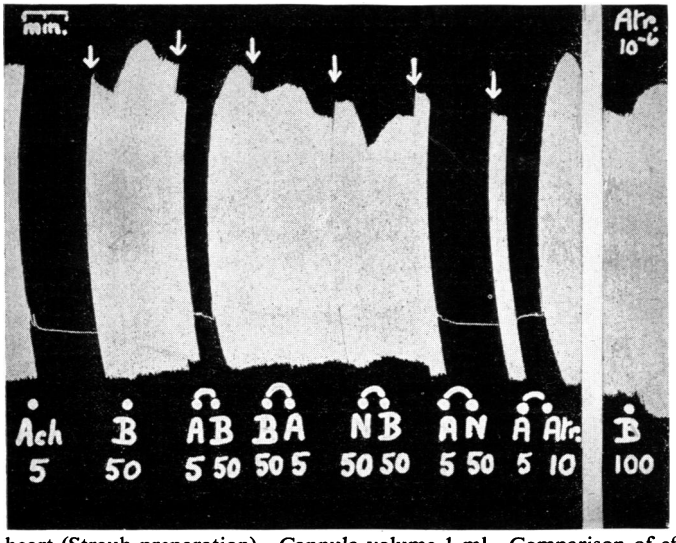

FIG. 5.—Frog heart (Straub preparation). Cannula volume 1 ml. Comparison of effects of acetylcholine (A), benzoylcholine (B), nicotine (N), and atropine (Atr.). Doses in μg. Time in min. Wash at arrows. Note that benzoylcholine stimulates the heart even in the presence of nicotine and atropine. In the presence of excess acetylcholine, benzoylcholine and atropine restart the heart.

DISCUSSION

Benzoylcholine has been widely used in enzyme studies as a substrate for pseudocholinesterase. It is freely soluble in water, and yet its other pharmacological actions appear to have been little studied. It possesses a structural formula which resembles those of many commonly used local anaesthetics, such as procaine and amylocaine, and yet we have found it to be lacking in this property. This is not surprising, since benzoylcholine is a quaternary base and exists in solution as stable cations and anions. On the other hand, most local anaesthetics contain a tertiary amino group, and activity appears to depend upon the concentration of undissociated base (Trevan and Boock, 1927). Bovet and his co-workers (1949) noted that benzoylcholine is a neuromuscular blocking agent in rabbits and is relatively non-toxic (LD50 in rabbits : 150 mg./kg.). We have now shown that it produces neuromuscular block in the rat diaphragm and cat gastrocnemius and paralysis in chicks. In chicks it produces spasticity so that benzoylcholine probably causes depolarization of the endplate region of the muscle fibre, like that produced by decamethonium or succinylcholine. It does not act like curare, for in the frog rectus muscle it has no action on the acetylcholine response but itself causes stimulation, which is blocked by curare and procaine. Its action is little affected by eserine.

On smooth muscle, benzoylcholine appears to exert at least three distinct actions. In small doses in the trachea preparations, it potentiates the acetylcholine response,

possibly by inhibition of the cholinesterase ; in medium doses in all preparations, it blocks the acetylcholine response, possibly by attaching itself to the same receptors ; and in larger doses, it stimulates most forms of smooth muscle, in part through a direct stimulant action.

The weak muscarine-like property of benzoylcholine is best shown on the blood pressure of a freshly anaesthetized animal. Repeated doses, however, usually result in the disappearance of the hypotensive response and the appearance of hypertension (nicotine-like action).

There is no doubt that benzoylcholine directly stimulates auricular and ventricular cardiac muscle, and it is the only choline derivative so far studied that will stimulate the heart in the presence of acetylcholine. The blocking action of benzoylcholine on acetylcholine is possibly the result of competitive antagonism, as was suggested in the smooth-muscle experiments, but further work on rabbit auricles suggests that at least five times as many molecules of benzoylcholine must be present for the usual acetylcholine response to be overcome. Benzoylcholine is known to be broken down in the body into benzoic acid and choline, but a solution of equimolar amounts of benzoic acid and choline does not exert the effects of benzoylcholine ; in fact, the mixture behaves like a solution of choline, so that it must be the benzoylcholine molecule itself which is responsible for the observed effects.

All the results suggest that benzoylcholine may be a useful tool for the study of the mechanism by which acetylcholine exerts its characteristic actions.

Summary

1. Benzoylcholine possesses strong nicotine-like properties and weak muscarine-like properties.

2. In addition, benzoylcholine has a direct stimulant action on gut and heart ; this action is unaffected by atropine.

3. Benzoylcholine restarts the frog heart and rabbit auricles after they have been stopped by excess of acetylcholine.

4. Benzoylcholine blocks the acetylcholine response on gut, trachea, and heart.

5. On the rabbit auricles, small ineffective doses of benzoylcholine block the response of effective doses of benzoylcholine.

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