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ACTION OF BRETYLIUM ON THE ISOLATED GUINEA-PIG ILEUM

BY

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Bretylium inhibits the contractions of the longitudinal muscle caused by histamine and, to a lesser extent, those by acetylcholine, carbachol and nicotine. It is a very weak antagonist of 5-hydroxytryptamine and in concentrations of up to 190 μg/ml. does not inhibit the actions of bradykinin and substance P on the longitudinal muscle. The inhibitory effect of bretylium on the emptying phase of the peristaltic reflex is similar to that of ganglion blocking agents, while the much less pronounced effect on the contraction of the longitudinal muscle during the preparatory phase is assumed to be due to a weak atropine-like action.

When Boura & Green (1959) reported their experiments on the inhibition of adrenergic neurones by bretylium, they described a number of other effects. One of these, the inhibitory effect on the peristaltic reflex in the isolated guinea-pig ileum, attracted our attention, as we thought that it might yield information on the nature of this reflex. Since adrenaline and noradrenaline depress the peristaltic reflex (Kosterlitz & Robinson, 1957), its inhibition by bretylium cannot be due to a block of adrenergic neurones.

Boura & Green (1959) also observed that the contractions of the longitudinal muscle of the ileum caused by histamine were reduced by bretylium but not those caused by acetylcholine or 5-hydroxytryptamine. These findings have been re-examined by us and extended to the contractions of the longitudinal muscle produced by other substances.

METHODS

The method of Trendelenburg (1917) was used for investigating the effect of bretylium on the peristaltic reflex of the isolated guinea-pig ileum. Contractions of the longitudinal muscle layer caused by addition of drugs to the bath fluid were recorded either isotonically by a conventional light lever or isometrically by a mechano-electrical transducer (Innes, Kosterlitz & Robinson, 1957).

The bath fluid was Tyrode solution with a low magnesium sulphate content (0.01 g/l.), oxygenated with 100% O_2. There were intervals of 3 min between the individual experimental procedures such as the eliciting of the reflex or the addition of drugs. The bath fluid was renewed at least every 6 min to prevent a significant rise of the *p*H above the initial value of about 8.

A segment of the distal end of the guinea-pig ileum was used after discarding the 10 cm nearest to the ileo-caecal valve. The ileum was set up with an initial tension of 1 *g* about 1 hr before the beginning of the experiment.

The doses of the drugs are given in weight of base for acetylcholine chloride, carbachol (carbaminoyl-choline chloride), histamine diphosphate, 5-hydroxytryptamine creatinine sulphate and nicotine bitartrate; in weight of salt for hexamethonium iodide and bretylium tosylate. Substance P and bradykinin were used as partly purified preparations; the doses of the latter are given in Roche e Silva units.

In confirmation of the findings of Boura & Green (1959), no difference was observed between the actions of bretylium tosylate and bretylium bromide.

RESULTS

Effect of bretylium on contractions of the longitudinal muscle caused by drugs

Acetylcholine, carbachol and histamine. Bretylium depressed the responses of the longitudinal muscle to all three substances, the minimum effective concentration being 2 μg/ml. The inhibitory effect, which reduced both the height and the speed of contraction, was observed within 3 min of the addition of bretylium to the bath; it was readily reversible by washing out the drug.

Bretylium antagonized the action of histamine more powerfully than that of acetylcholine (Fig. 1). When the ratios of the doses causing equal contractions in

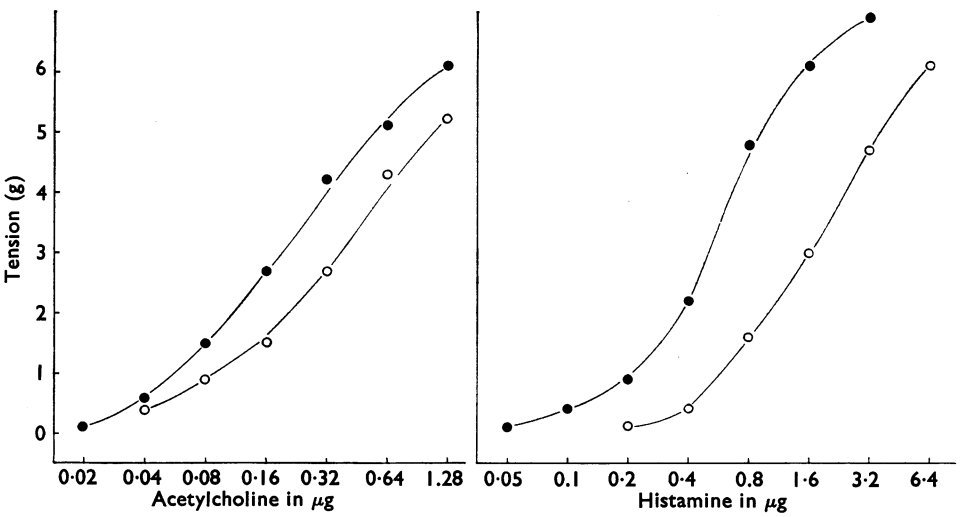

Fig. 1. Dose-response curves for acetylcholine and histamine alone (●——●) and in the presence of bretylium tosylate 14 μg/ml. bath fluid (○ —— ○). Abscissa: doses of acetylcholine or histamine added to 40 ml. bath. Ordinate: tension developed in isometric contractions of the longitudinal muscle.

the presence (14 μg/ml.) and absence of bretylium were calculated, they were found to be 4.3 in the case of histamine and 2 in the case of acetylcholine.

5-Hydroxytryptamine and nicotine. Drugs which cause contraction of the longitudinal muscle by stimulation of neuronal elements were also antagonized by bretylium. In a concentration of 18 μg/ml., bretylium depressed the action of nicotine but not that of 5-hydroxytryptamine. The smallest concentration of bretylium which had an inhibitory effect on the contraction due to 5-hydroxytrypt-

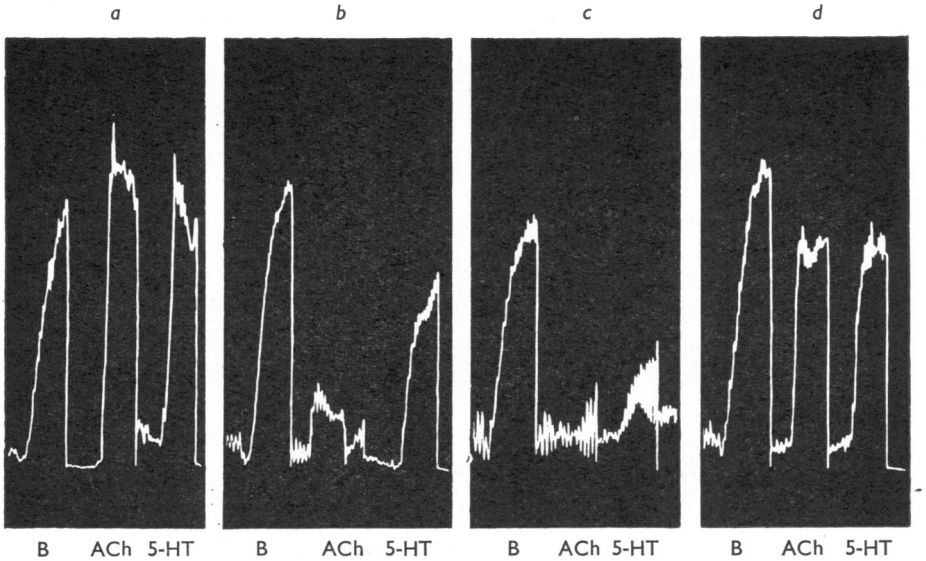

Fig. 2. The effect of bretylium on the contractions of the longitudinal muscle caused by bradykinin, acetylcholine and 5-hydroxytryptamine. Bath containing 7·5 ml. Tyrode solution. (*a*) Controls; (*b*) 5 min after addition of bretylium tosylate (100 μg/ml. bath fluid) ; (*c*) 14 min after increasing the bretylium concentration to 190 μg/ml.; (*d*) 12 min after washing out bretylium. B: 0·5 units bradykinin; ACh: 10 ng acetylcholine; 5-HT: 0.2 μg 5-hydroxytryptamine.

amine was 100 μg/ml. (Fig. 2). The ratio of doses of nicotine having equal potency in the presence (11 μg/ml.) and absence of bretylium was 1.5.

Bradykinin and substance P. The actions of these two polypeptides were not inhibited by bretylium in concentrations up to 190 μg/ml. In these large doses, bretylium itself caused a transient contraction of the longitudinal muscle, an observation already made by Boura & Green (1959). When the bretylium was left in the bath, the muscle relaxed again after 3 to 5 min and for a further period of about 10 min remained insensitive to the stimulating drugs used, an effect similar to that described by Cantoni & Eastman (1946). Thereafter the responses to the polypeptides returned to normal while those to acetylcholine, histamine and 5-hydroxytryptamine remained depressed. For instance, in the presence of 100 to 190 μg/ml. of bretylium, the contractions due to acetylcholine and 5-hydroxytryptamine were markedly reduced, while those caused by bradykinin were affected only a little, if at all (Fig. 2).

Effect of bretylium on the peristaltic reflex elicited by distension of the lumen

The inhibitory effect of bretylium was more marked on the second or emptying phase of the reflex than on the first or preparatory phase. The contractions of the circular muscle during the emptying phase were abolished by concentrations of 5 to 18 μg/ml., which had no effect on the contractions of the longitudinal muscle layer during the preparatory phase (Fig. 3).

These experiments were repeated after blocking the emptying phase with hexamethonium (25 μg/ml.). Again, bretylium in concentrations of 5 to 18 μg/ml. had

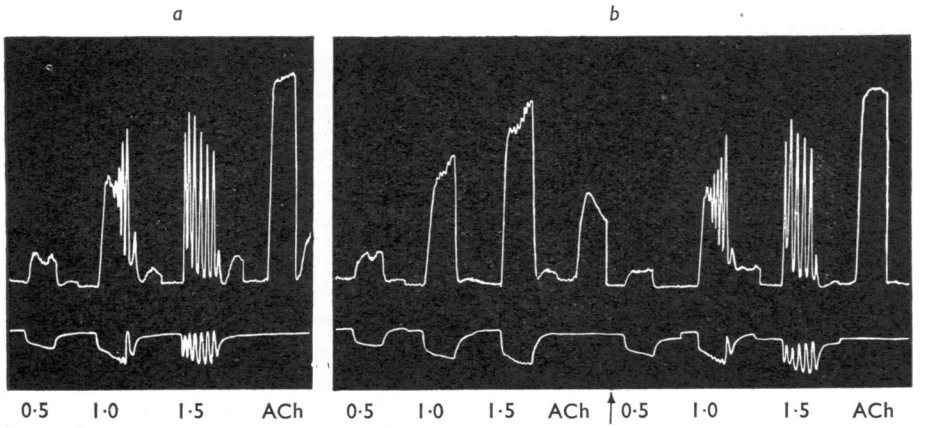

Fig. 3. The effect of bretylium on the peristaltic reflex. 40 ml. bath. Upper tracing: contraction of longitudinal muscle; lower tracing: filling of intestinal lumen. (*a*) Controls; (*b*) 15 min after addition of bretylium tosylate (15 μg/ml. bath fluid), washed out at arrow. The figures below the tracings indicate intra-intestinal pressures in cm of water. ACh: 0.1 μg acetylcholine.

no inhibitory effect on the contraction of the longitudinal muscle during the preparatory phase, although the responses to acetylcholine, carbachol and histamine were reduced. When the concentration of bretylium was raised beyond 100 μg/ml. the preparatory phase was partly inhibited, the contraction due to acetylcholine (1.3 ng/ml.) almost completely abolished while that due to bradykinin was scarcely affected (Fig. 4).

The inhibitory effect of bretylium on the emptying phase was observed within 3 min of the addition of the drug to the bath fluid ; recovery took place within 3 min after washing it out. With the larger doses required for inhibition of the longitudinal contraction during the preparatory phase, recovery was delayed for 20 to 30 min.

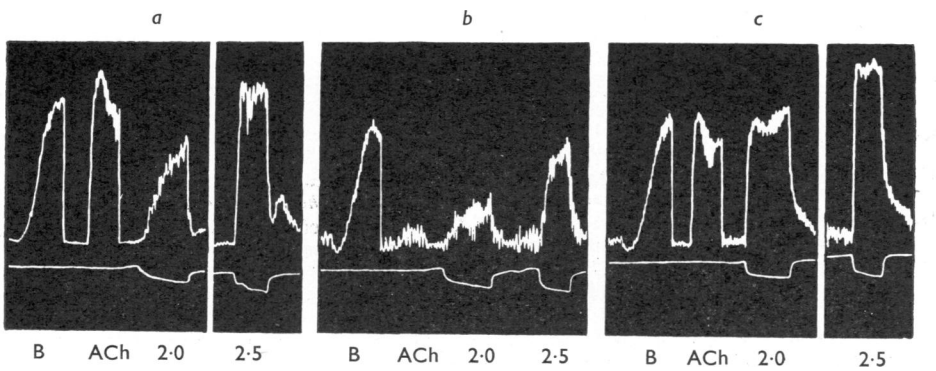

Fig. 4. The effect of bretylium on the peristaltic reflex in the presence of hexamethonium (25 μg/ml. bath fluid). Bath containing 7.5 ml. Tyrode solution. Upper tracing: contraction of longitudinal muscle; lower tracing: filling of intestinal lumen. (*a*) Controls; (*b*) 16 min after addition of bretylium tosylate (190 μg/ml. bath fluid); (*c*) 31 min after washing out bretylium. The figures below the tracings indicate intra-intestinal pressures in cm of water. B: 0.35 units bradykinin; ACh: 10 ng acetylcholine.

DISCUSSION

Our results confirm the findings of Boura & Green (1959) as far as the inhibitory effect of bretylium on the peristaltic reflex and on the contraction of the longitudinal muscle due to histamine is concerned. We find, in addition, that bretylium antagonizes, although to a lesser extent, acetylcholine, carbachol and nicotine, and that the inhibitory effect on the acetylcholine and carbachol contractions is also observed in the presence of hexamethonium. These findings suggest that bretylium has a weak atropine-like action.

The reversible inhibitory effect of low concentrations of bretylium (5 to 20 μg/ml.) on the peristaltic reflex is similar to that found with ganglion-blocking agents, in that it affects only the emptying phase and not the shortening of the gut during the preparatory phase (Feldberg & Lin, 1949; Paton & Zaimis, 1949; Kosterlitz & Robinson, 1957). The onset of the block of the cholinergic neurones is rapid and recovery is quickly brought about by washing out the drug. Boura & Green (1959) had already found that bretylium causes a readily reversible ganglionic block in the perfused superior cervical ganglion of the cat, and stressed that this is very different from the slow development and long persistence of the block which bretylium produces in adrenergic neurones.

The fact that high concentrations of bretylium (100 to 200 μg/ml.) inhibit the contractions of the longitudinal muscle during the preparatory phase is not necessarily in disagreement with the view that the inhibition of the emptying phase is due to a ganglion-blocking action. While the reflex arc of the preparatory phase does not contain cholinergic synapses, the transmission at the neuro-effector junction is inhibited by atropine (Schaumann, 1955; Kosterlitz & Robinson, 1957, 1959). It may therefore be assumed that the inhibition of the preparatory phase by high concentrations of bretylium is due to its weak atropine-like action.

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