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THE GANGLION BLOCKING ACTION OF PROCAINAMIDE

BY

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In cats and rabbits procainamide (20 to 50 mg, intravenously) produced a fall of blood pressure of 20 to 50 mm Hg which reached a maximal effect within 1 min and lasted for about 5 min. Procainamide reduced the pressor responses to nicotine and to carotid arterial occlusion and reduced the depressor response to vagal stimulation, but did not antagonize the actions of adrenaline or noradrenaline on blood vessels. The contractions of the nictitating membrane to stimulation of the preganglionic cervical sympathetic nerve were partially or completely blocked by 20 to 50 mg of procainamide given intravenously. The ganglion blocking effect was more abrupt in onset and more slow to recover than that due to hexamethonium and had about 1/250th of the activity of the latter. Procainamide (1 mg) reduced the acetylcholine output of the perfused superior cervical ganglion to below 30% of the control value and blocked transmission completely. Small doses (10 μg) reduced the acetylcholine output but hardly affected ganglionic transmission. Procainamide, injected into the perfused superior cervical ganglion, blocked contractions elicited by stimulation of the preganglionic cervical sympathetic nerve for a longer period than those produced by acetylcholine injected into the perfusion circuit to the ganglion; the reverse was true for hexamethonium. Procainamide reduced the size of action potentials recorded from the superior cervical ganglion without altering the resting potential of the ganglion. The ganglion blocking activities of procainamide and hexamethonium often potentiated each other, especially when the preparation had been set up for several hours. On the guinea-pig isolated ileum preparation, procainamide (0.5×10^{-4} g/ml.) antagonized responses due to acetylcholine, histamine and, most effectively, to nicotine. On the isolated heart, procainamide (1 mg) almost abolished the bradycardia produced by acetylcholine; 10 mg slowed and weakened the heart, while 100 mg stopped it. We conclude that procainamide, like procaine, blocks ganglionic transmission by (1) depressing the release of acetylcholine from preganglionic nerve endings; and (2) competing, with the acetylcholine which is released, for receptor sites on the ganglion cells. The amounts required to produce significant effects *in vivo* and *in vitro* are comparable. The methods available for detecting this type of ganglion blocking action are discussed.

The use of procainamide in the treatment of cardiac arrhythmias is now well established, although its action is still incompletely understood. Another action, that of the ganglion block, is, however, less generally recognized. The possibility of such an action was suggested by G. E. H. Enderby (personal communication) who observed that procainamide considerably increased the hypotension produced by hexamethonium and tilt, thus making it possible to obtain "bloodless field" surgery more easily. Since procaine was already known to interfere with ganglionic

transmission, this observation suggested that procainamide shared such an action and could synergize with hexamethonium.

Our experiments have been directed, therefore, to testing the ganglion blocking properties of procainamide and their mechanism. A preliminary account has been published (Paton & Thompson, 1953). During the course of our experiments the paper by Reuse & Bergmann (1952), which describes some similar results in the dog, came to our notice.

METHODS

Cats and rabbits were anaesthetized with chloralose (80 mg/kg), given intravenously after induction with ethyl chloride and ether. A tracheal cannula was always inserted. Blood pressure was recorded with a mercury manometer through a siliconed cannula containing heparinized saline in one carotid artery. Injections were given through a cannula in the femoral vein.

To stimulate a vagus, platinum electrodes were placed on its distal part after separation from the cervical sympathetic nerve and division in the neck; supramaximal stimuli, at 10 shocks/sec, were used. In a few early experiments the vagus and other nerves were stimulated with supramaximal condenser shocks, but in the majority of experiments the nerves were stimulated with supramaximal rectangular shocks of 0.5 msec duration. Carotid arterial occlusion was carried out by clipping the uncannulated carotid artery with a "bulldog" clip for a period of 30 sec.

The superior cervical ganglion was perfused by the method of Kibjakow (1933) and Feldberg & Gaddum (1934), with MacIntosh's modification for warming the perfusion fluid by passing the polyethylene supply-tube through the stomach and oesophagus. Locke solution or that employed by Edlund & Lohi (1952) was used, with the addition of physostigmine or neostigmine (2 μg/ml.). Drugs were made up in the solution used for perfusion and injected into the arterial cannula through a Gordh needle thrust well down into its side-arm. The cervical sympathetic trunk was excited with supramaximal stimuli at 10 shocks/sec. The contractions of the nictitating membrane were recorded on a smoked drum, after enucleation of the eyeball and excision of the eyelids. The samples of perfusate collected were frozen until they were assayed for acetylcholine content on the blood presssure of an eviscerate cat.

Ganglion action potentials were recorded by the method of Paton & Perry (1953), using nonpolarizable electrodes and DC amplification. During these experiments injections were made either intravenously or close-arterially into the stump of the external carotid artery.

Respiration was recorded on smoked paper with an integrating minute volume recorder (Paton, 1949).

The rabbit isolated heart with intact vagus nerves was perfused by the method of Hoffmann, Middleton & Talesnik (1945) using Tyrode solution. The heart movements were recorded on a smoked drum by means of a spring-loaded lever.

The guinea-pig ileum preparation was set up in the usual way in oxygenated Tyrode solution at 34° C, in an organ-bath of 20 ml. capacity.

The following drugs were used: procainamide hydrochloride, hexamethonium bromide, acetylcholine iodide, nicotine acid tartrate, adrenaline and noradrenaline hydrochlorides and histamine acid phosphate. Doses of histamine and adrenaline are expressed as bases, the remainder as salts.

RESULTS

Experiments on the whole animal

Eight experiments were made with intravenous injections into cats anaesthetized with chloralose. Procainamide produced a fall in blood pressure depending less

on the dose than on the animal. Thus 20 mg elicited a fall of only 20 mm Hg in one experiment, and 55 mm Hg in another; 50 mg lowered the blood pressure by 25 to 50 mm Hg. In one experiment, in which the blood pressure was low, 100 mg produced only a transient fall in pressure of about 25 mm Hg. The fall in blood pressure was usually rapid, reaching its peak within 1 min and lasting about 5 min; there was no great change of heart rate.

This fall in blood pressure was not due to an antagonism to adrenaline or noradrenaline on the blood vessels. Fig. 1 illustrates how 5 μg of adrenaline was, if anything, somewhat potentiated by 100 mg of procainamide, and similar results were obtained with smaller doses of the amide. The action of noradrenaline was only slightly impaired in the experiment of Fig. 1, but in other experiments it was

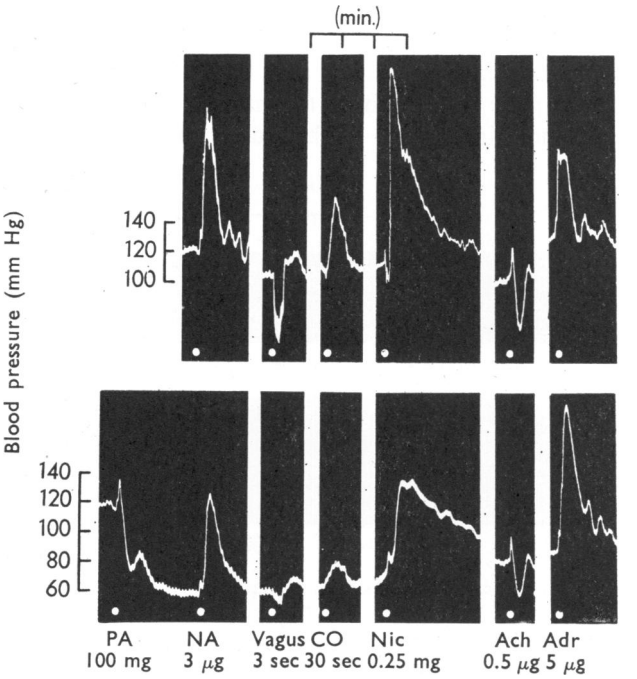

Fig. 1. Cat, chloralose anaesthesia. Responses of blood pressure to intravenous injections of noradrenaline (NA, 3 μg), nicotine (Nic, 0.25 mg), acetylcholine (Ach, 0.5 μg), adrenaline (Adr, 5 μg), and to vagal stimulation for 3 sec and carotid arterial occlusion (CO) for 30 sec before (above) and after (below) administration of 100 mg of procainamide (PA). Blood pressure in mm Hg; time scale in minutes.

somewhat augmented. Similar observations were made in an experiment in the rabbit anaesthetized with chloralose.

On the other hand, procainamide (10 to 100 mg) could reduce or abolish the response to nicotine (0.1 to 0.5 mg) (Fig. 1). Out of six experiments on cats, once, with a dose of 20 mg of procainamide, the bradycardia produced by nicotine was abolished and the pressor effect slightly increased; but with higher doses, nicotine's pressor action was always attenuated. The response to stimulating the vagus nerve

was modified; sometimes it was simply attenuated, sometimes the rapid onset of bradycardia and hypotension was slowed rather than reduced in amplitude. In addition, the pressor response to carotid arterial occlusion was much reduced. These effects point to a ganglionic site for the hypotensive action of procainamide.

During these experiments it was also noticed that procainamide reduced the absolute magnitude of the depressor effect of acetylcholine in a dose of 0.5 μg and that of histamine in the same dose; but since procainamide itself changed the level of the blood pressure, only a strong antagonism could have been regarded as significant. Procainamide affected respiration if given in large doses; 100 mg in a cat anaesthetized with chloralose reduced both minute volume and respiration rate by about half. In one experiment an initial transient stimulation occurred.

Experiments on the superior cervical ganglion

In seven experiments on cats, procainamide (20 to 50 mg intravenously) produced partial or complete relaxation of the nictitating membrane excited by preganglionic sympathetic stimulation. The quick spontaneous movements of the

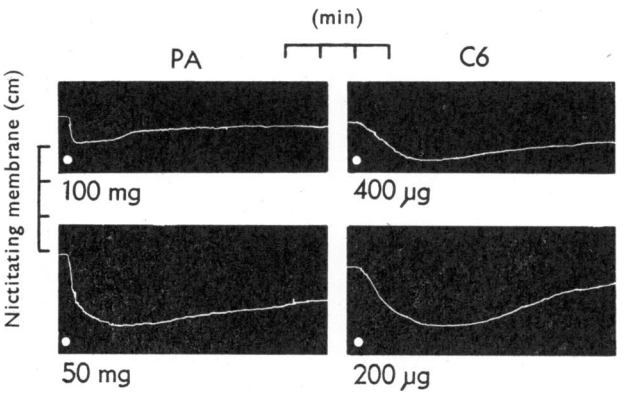

Fig. 2. Cat, chloralose anaesthesia. Record of the relaxations of the nictitating membrane which were produced by intravenous injections of 100 and 50 mg of procainamide (PA) and 400 and 200 μg of hexamethonium (C6). The preganglionic cervical sympathetic nerve was stimulated supramaximally at 10 shocks/sec throughout. Vertical scale in cm; time scale in minutes. The lower records were obtained 6.5 hr after the upper records, during which time preganglionic excitation continued, and illustrate the resulting increase in sensitivity of the preparations.

nictitating membrane, often seen when anaesthesia became light, were not affected. The time course of action differed from that due to hexamethonium; the response with procainamide was more abrupt in onset, and slower to recover, than with hexamethonium, even when the amplitude of the response was smaller (Fig. 2). Procainamide was much less potent than hexamethonium, having about 1/250th of the activity of the latter.

Acetylcholine output. To analyse this action further, two experiments on the perfused superior cervical ganglion were undertaken. The perfusion fluid contained physostigmine, so that acetylcholine output by the ganglion could be measured. Procainamide (1 mg, intra-arterially) blocked transmission completely, and responses

of the nictitating membrane to preganglionic excitation began to return only after 30 min. During this time, the acetylcholine output fell from 9 mμg per stimulation period (10 shocks/sec for 3 min) to undetectable levels (less than 3 mμg), and then recovered as transmission was restored.

In the other experiment with 10 μg of procainamide (Fig. 3), acetylcholine output was again depressed, from 34 to 18 mμg, but the interference with transmission was hardly detectable. Evidently output could be significantly reduced before trans-

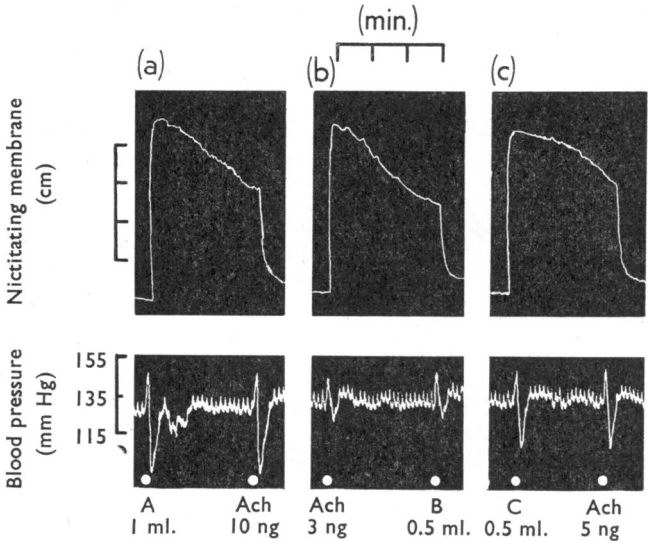

Fig. 3. Cat, chloralose anaesthesia. Upper record: perfusion of right superior cervical ganglion. The record shows contractions of the right nictitating membrane in response to supramaximal stimulation of the preganglionic cervical sympathetic nerve at 10 shocks/sec for periods of 3 min. (*a*) control; (*b*) after procainamide (10 μg) had been injected into the perfusion circuit, 1.25 min before stimulation began; (*c*) control. The perfusate was collected as a continuous series of samples. Each sample was collected over a period which began just before the onset of stimulation and in (*a*), (*b*) and (*c*) lasted for 25, 29 and 52 min respectively. Lower record: assay of acetylcholine. The record shows blood pressure responses of an eviscerated cat (treated with physostigmine) to intravenous injections of acetylcholine (Ach) and of samples A, B and C of perfusate obtained during and after the corresponding periods of stimulation (*a*), (*b*) and (*c*) shown in the upper record. The lower record shows only matched responses, but during the assay the response to each sample was straddled by responses to acetylcholine which were slightly smaller and slightly larger than the response to the sample. Calibrations: horizontal in minutes; vertical in cm (upper) and mm Hg (lower).

mission began to fail, an interesting aspect of the high safety factor for transmission in the ganglion.

Antagonism to injected acetylcholine. In addition to depressing acetylcholine release in the ganglion, procainamide antagonized the stimulant action of acetylcholine on the ganglion cells, just as does procaine (Harvey, 1939). This leads to a contrast with hexamethonium (Fig. 4). With the latter, block of the response to injected acetylcholine was more easily produced and lasted longer than block of

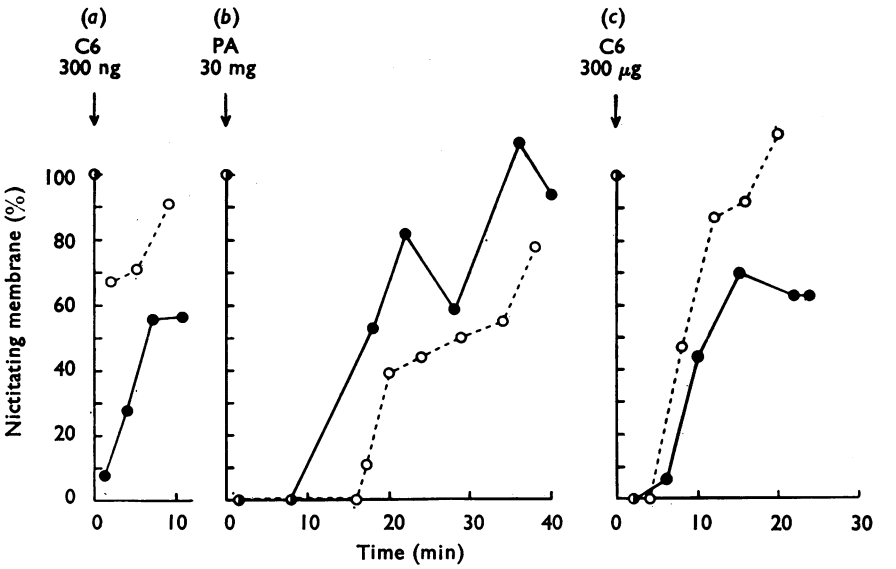

Fig. 4. Cat, chloralose anaesthesia. Graphs of contractions of a nictitating membrane expressed as a percentage of controls. Retrograde arterial injections into the right lingual artery with the external carotid artery occluded distal to the lingual artery during, and for 10 sec after, injection. Contractions were produced by supramaximal stimulation of the preganglionic cervical sympathetic nerve for 10 sec, at 3 shocks/sec (○---○), and by injection of acetylcholine (80 μg) into the ganglion perfusion circuit (●——●). In (*a*) hexamethonium, (C6) 300 μg; (*b*) procainamide (PA), 30 mg; and (*c*) a second dose of hexamethonium, 300 μg, were injected. Ordinate, height of contraction expressed as percentage of control value; abscissa, time in minutes. The control response to acetylcholine was within 10% of the same magnitude as the control response to stimulation of the cervical sympathetic nerve.

the response to preganglionic nerve stimulation. But with procainamide, it was the response to preganglionic excitation which was the more vulnerable. In the second test with hexamethonium (Fig. 4 *c*), the response to nerve stimulation was proportionately more reduced than in the first test (Fig. 4 *a*); although the point was not explored further, this result was probably due to the persistence of procainamide given previously (Fig. 4 *b*).

Ganglion action potentials. Procainamide differs from other ganglion-blocking agents: from nicotine by its lack of stimulant action, and from hexamethonium and similar drugs, as well as nicotine, by its effect on acetylcholine release. It was desirable therefore to test electrophysiologically its action on the ganglion cell, and this was done in two experiments. The procainamide was injected intravenously. A dose of 10 mg had a trivial effect; but 100 mg reduced the amplitude of the action potential elicited by single shocks by about half (Fig. 5). At no time was a change in the resting potential produced. An interesting fact was that the shape of the action potential, during partial block, differed from that seen with hexamethonium. Characteristically with the latter the positive afterpotential increased somewhat as block came on; but with procainamide the whole complex became reduced evenly in all its components.

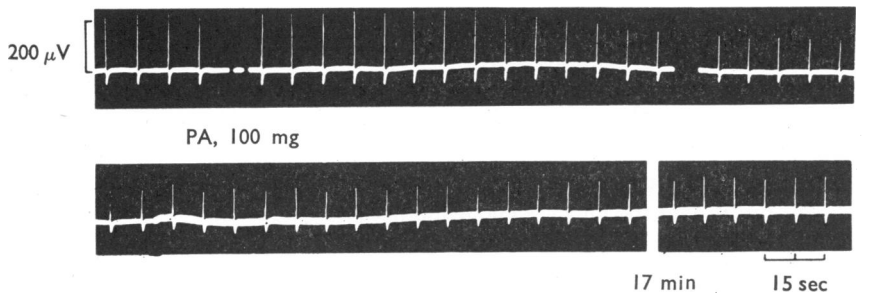

Fig. 5. Cat, chloralose anaesthesia. Record of ganglion action potentials recorded from right superior cervical ganglion; the record reads from left to right. The preganglionic cervical sympathetic nerve was stimulated supramaximally at 4 shocks/min. Procainamide (PA), 100 mg, was injected intravenously at the first gap in the upper record. (The preparation was inspected at the second gap in the upper record.) The first (left) part of the lower record was continuous with the end of the upper record; the second (right) part of the lower record was taken after an interval of 17 min. Vertical scale, 200 μV; time scale, 15 sec.

Synergism with hexamethonium. It is clear from the results already mentioned that procainamide is of relatively low activity at the ganglion, and it may be questioned whether its activity is sufficient to be of any practical importance. But since it was its use in conjunction with hexamethonium that originally interested us, a more relevant question is that of its activity together with the latter drug. The combination is also of some interest because it links a drug depressing release with one competitively antagonizing acetylcholine.

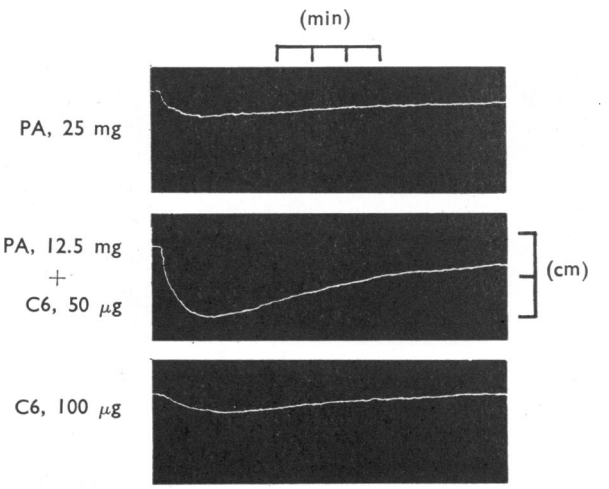

Fig. 6. Cat, chloralose anaesthesia. Record of the relaxations of the nictitating membrane which were produced by intravenous injections of procainamide (PA), 25 mg (upper record), a mixture of procainamide, 12.5 mg, and hexamethonium (C6), 50 μg (middle record), and hexamethonium, 100 μg (lower record). The preganglionic cervical sympathetic nerve was stimulated supramaximally at 10 shocks/sec throughout. Vertical scale in cm; time scale in minutes.

Fig. 6 shows how, in fact, the two drugs can interact. 25 mg of procainamide and 100 μg of hexamethonium were each given separately, and the responses to these injections were compared with that to a mixture of half of each of these doses. If the two drugs were simply additive, then the resulting response should have lain between that due to 25 mg of procainamide and that due to 100 μg of hexamethonium alone; if there was some mutual antagonism, the mixture should have had less effect; but in fact the effect was considerably increased, indicating that when the drugs were given together there could be a mutual potentiation.

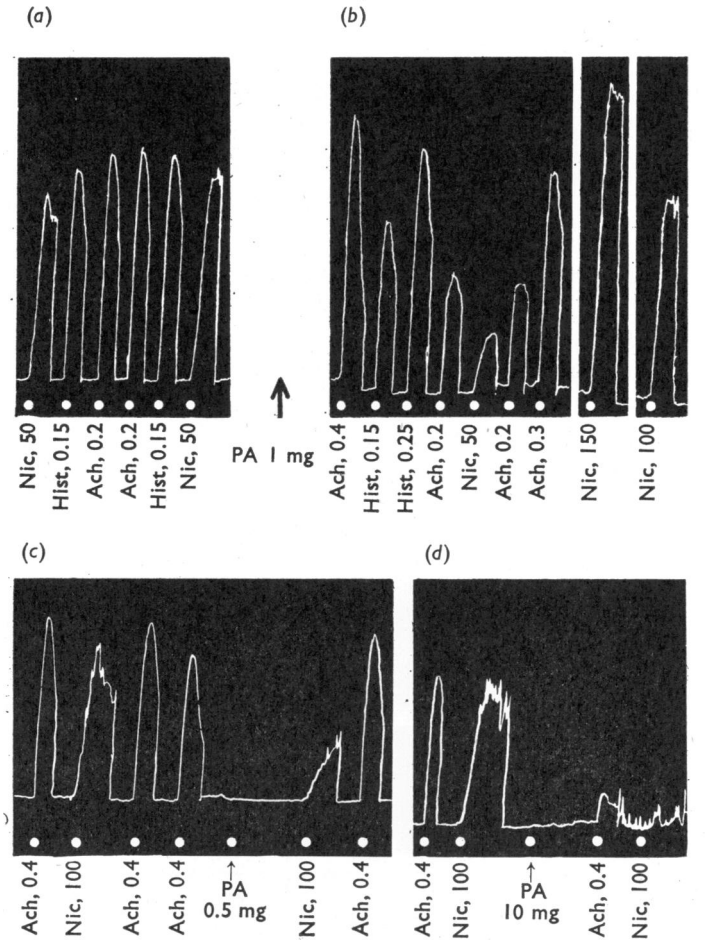

Fig. 7. Guinea-pig ileum preparation. Two experiments, upper and lower records. Contractions were produced by adding nicotine (Nic), histamine (Hist) and acetylcholine (Ach) in the doses shown (μg) to a 20 ml. capacity organ-bath. First experiment: contractions obtained (*a*) before, and (*b*) in the presence of procainamide (PA), 1 mg in the bath. Second experiment: the effects of procainamide, 0.5 mg in (*c*) and 10 mg in (*d*), on the contractions produced by adding acetylcholine and nicotine. Drugs were added to the bath, with a 1 min cycle, at the white dots: exposure to stimulants was for 15 sec, save in (*c*) and (*d*) when exposures to nicotine were for 30 sec; the drum was stopped at washouts for 15 or 30 sec.

This potentiation was not always seen, and it seemed to be most obvious when the preparation had been set up for several hours. When it appeared, it was approximately quantitated with the aid of dose/response curves which proved to be parallel for the two drugs separately. The dose/response curves were obtained from a comparison of the two drugs at two dose levels, using a randomized Latin Square design. For instance, in one such test, 100 μg of hexamethonium given with 12.5 mg of procainamide produced a relaxation corresponding to that resulting from 275 μg of hexamethonium or from 62 mg of procainamide. On the basis of a simple addition of effects, the equivalent doses should have been 155 μg of hexamethonium and 35 mg of procainamide. The ratio of the observed equivalent dose to the expected equivalent dose (reduced to terms of either drug, no matter which) was thus approximately 1.77, a potentiation of 77%. In the early stages of a preparation, before potentiation was obvious, effects were simply additive.

Experiments on isolated organs

Guinea-pig ileum. Ganglion blocking drugs can be recognized, on the guinea-pig ileum for instance, by their ability to antagonize a ganglion stimulant such as nicotine (Feldberg, 1951). The test becomes more difficult if the drug is also an antagonist to the postganglionic transmitter, acetylcholine, but a differential action may be demonstrable. Procainamide is such a compound. Fig. 7, *a* and *b*, illustrates an experiment in which, in a concentration of 0.5×10^{-4} gm/ml., procainamide antagonized not only nicotine but also acetylcholine and histamine. The antagonism, however, was greatest against nicotine, and least against histamine. From the dose-ratios obtained in this and other experiments, the concentrations of procainamide for a twofold antagonism against nicotine, acetylcholine and histamine were estimated (Paton, 1961) respectively as approximately 5×10^{-5}, 1 to 1.5×10^{-4} and 1.5 to 2×10^{-4} gm/ml. With careful choice of dose, it was possible to produce a substantial reduction of the response to nicotine with only little effect on that to acetylcholine, although a higher dose of procainamide would affect the latter also (Fig. 7, *c* and *d*).

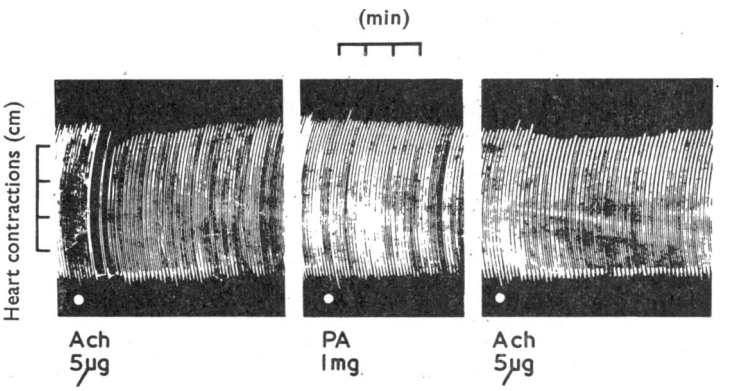

Fig. 8. Contractions of isolated perfused rabbit heart. At the white dots, injections of acetylcholine (Ach), 5 μg (left record), procainamide (PA), 1 mg (middle record), and a second dose of acetylcholine, 5 μg (right record), were made into the perfusate. Vertical scale in cm; time scale in minutes.

Rabbit heart. The action of procainamide was tested in one experiment on a perfused rabbit heart. A dose of 1 mg of procainamide injected into the perfusion cannula had no effect itself on the heart, but almost abolished the bradycardia produced by an injection of 5 μg of acetylcholine (Fig. 8). A larger dose, 10 mg, of procainamide itself slowed and weakened the heart, and the augmentor effect of 0.5 μg of adrenaline was now greatly reduced. A very large dose, 100 mg, of procainamide stopped the heart at once.

DISCUSSION

Reuse & Bergman (1952) showed, in the dog, that procainamide, in addition to possessing its well-known action on cardiac arrhythmias, could block the response of the heart to vagal stimulation and of the blood pressure to carotid arterial occlusion or splanchnic nerve stimulation, and that it was itself hypotensive. They compared its action with that of procaine, shown by Harvey (1939) to interfere with ganglionic transmission. The experiments described in this paper confirm these results, but in the cat. The evidence that procainamide interferes with the ganglionic synapse is as follows: it produces a hypotension not explained by antagonism to noradrenaline nor by an action on the heart; it reduces the pressor response to nicotine or to carotid arterial occlusion, and the bradycardia to vagal stimulation; it blocks the response of the nictitating membrane to preganglionic stimulation of the cervical sympathetic nerve, both in the whole animal and with a perfused ganglion, and depresses the ganglion action potential; on the guinea-pig isolated ileum preparation it preferentially antagonizes nicotine. It is, however, far from specific, and at somewhat higher doses antagonizes acetylcholine, histamine and adrenaline under various conditions.

The potency of procainamide in interfering with ganglionic transmission by different tests appears to be fairly consistent. To produce a twofold antagonism to nicotine on the ileum, a concentration of about 50 μg/ml. was required. If one takes the estimate by Gray & Paton (1949) that an intravenous injection into a cat anaesthetized with chloralose and about 3 kg in weight becomes dissolved, after a few circulation times, in a volume of about 200 ml., it would be necessary to give approximately 10 mg of procainamide to produce readily detectable effects. This value agrees well with the results from all the experiments on the whole animal. We failed with a dose of 10 mg to affect the pressor response to stimulating the splanchnic nerve and it is interesting that Reuse & Bergmann (1952) also had difficulty in demonstrating an effect and required a dose of 40 mg/kg to obtain a definite result. This finding corresponds with the observation by Marley & Paton (1961) that the splanchnic nerve-suprarenal medullary synapse, stimulated intermittently in the normal manner, is rather resistant to ganglion blocking agents. With the perfused ganglion, the concentration of procainamide to which the ganglion was exposed is uncertain, since single injections into the perfusion stream were used. The threshold for block of transmission was obtained with an injection of 0.2 ml. of a 50 μg/ml. solution of procainamide.

The mechanism of ganglion block is the same as that demonstrated by Harvey (1939) for procaine. The release of acetylcholine is attacked, as well as the effective-

ness of the released acetylcholine in stimulating the ganglion cells. This double action may be responsible for the small but distinct differences from ganglion blocking agents such as hexamethonium. The acute onset of action of procainamide, the proportionately greater blocking action against the nerve-elicited response as compared with that against the response to injected acetylcholine, and the type of change of action potential shape, are among such differences. The mutual potentiation between procainamide and hexamethonium may also depend on the fact that procainamide brings in a second action, that of interference with acetylcholine release, especially since this potentiation appeared after a relatively prolonged period of preganglionic excitation when acetylcholine output might have fallen. These differences may, indeed, be characteristic of a ganglion blocking agent which interferes with acetylcholine release. But until more drugs of this type have been studied, it would be dangerous to rely on such differences, and it would still be essential to test for an action on acetylcholine release directly.

We are indebted to Dr J. Talesnik for showing us the method of perfusing the rabbit isolated heart. We should like to thank Mr John Haynes for his help in the preparation of the Figures. We are grateful to Mr R. Gartside (Squibb Ltd.) for a gift of procainamide (Pronestyl). Some of the earlier experiments which are reported in this paper were carried out in the Department of Applied Pharmacology, Medical Unit, University College Hospital Medical School, London.

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