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POTENTIATION OF SOME CATECHOL AMINES BY PHENOXYBENZAMINE, GUANETHIDINE AND COCAINE

BY

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In rabbit isolated atria, cocaine, guanethidine and phenoxybenzamine increased the changes in rate and force of contractions caused by noradrenaline and adrenaline, but did not potentiate isoprenaline. The most likely explanation for this result is that the drugs interfere with one of the mechanisms in the heart by which noradrenaline and adrenaline, but not isoprenaline, are inactivated; a probable mechanism would be the uptake of noradrenaline into storage sites in the tissue. Cocaine, guanethidine and phenoxybenzamine did not potentiate noradrenaline and adrenaline acting on the rabbit isolated duodenum and the rat uterus.

The experiments reported in this paper were suggested by the incidental observation that phenoxybenzamine reduced the threshold dose of adrenaline on isolated atria, while leaving the threshold to isoprenaline unaltered. This observation raised the question whether other drugs, such as cocaine and guanethidine, which enhance some actions of noradrenaline and adrenaline, would also potentiate isoprenaline. This problem was investigated using three isolated tissues—the rabbit atria and duodenum, and the rat uterus—in which noradrenaline, adrenaline and isoprenaline produced qualitatively similar results. Most of the other published experiments on drugs that potentiate the catechol amines have been concerned with the excitatory actions of adrenaline and noradrenaline, and consequently isoprenaline has not been included. The results described here show that phenoxybenzamine, cocaine and guanethidine enhanced the actions on rabbit atria of noradrenaline more than of adrenaline, while the response to isoprenaline remained unchanged. No potentiation of noradrenaline or adrenaline acting on rabbit duodenum or rat uterus could be demonstrated.

METHODS

Rabbit isolated atria. Isolated atria were suspended in a solution of the following composition (mM): Na^+ 145; K^+ 5.9; Ca^{++} 1.7; Mg^{++} 1.2; Cl^- 128; HCO_3^- 25; $H_2PO_4^-$ 1.2; $SO_4^=$ 1.2; and dextrose 5.6. The solution was gassed with 95% oxygen and 5% carbon dioxide. The volume of the organ-bath was 60 ml. and the temperature was maintained between 30 and 31° C. The atria were attached to a spring lever and their contractions were recorded on a smoked drum. In some experiments, the lever was arranged to interrupt a beam of light falling on a Mullard photoelectric cell, which recorded each atrial contraction on a decatron scaler. In other experiments, the rate of beating was counted using a stop-watch. In either instance, the rate was determined from counts made for 30 sec periods; for the

2 min before and the 3 min after the addition of a catechol amine to the organ-bath, the rate was determined at 1 min intervals. Catechol amines were added every 15 min and left in contact with the atria for 3 min. The maximal increase in heart rate, which usually occurred in the 2nd min, was taken as a measure of the response. In each experiment, the responses of the atria to 2 to 4 submaximal doses of two catechol amines were recorded, before and in the presence of a potentiating drug. The degree of potentiation was calculated from the horizontal displacement of the dose/response line in the presence of the potentiating drug.

Rat isolated uterus. The method was similar to that described by Gaddum, Peart & Vogt (1949); automatic assay apparatus was not used. Contractions of an isolated horn of a rat uterus were elicited by the addition of acetylcholine (about 2 μg) at 2 min intervals to the 15 ml. organ-bath. Noradrenaline (0.1 to 0.8 μg) or adrenaline (0.5 to 4 ng) was added 1 min before every third contraction.

Rabbit isolated duodenum. Segments of duodenum were suspended in 25 ml. of Tyrode solution, or of a solution of composition the same as that used for the atria, at 35° C, and attached to a light isotonic lever arranged to write on a smoked drum. Noradrenaline, adrenaline or isoprenaline was added at 10 min intervals and washed out after 40 or 50 sec.

Drugs. These were: (−)-adrenaline (Light); (−)-noradrenaline bitartrate (Light); (−)-isoprenaline sulphate (Wyeth); dopamine (Light); acetylcholine bromide (B.D.H.); phenoxybenzamine (Smith, Kline & French); guanethidine sulphate (Ciba); cocaine hydrochloride (B.D.H.); gallamine triethiodide (May & Baker); and hexamethonium bromide (May & Baker). Throughout, doses are expressed in terms of the bases.

RESULTS

Rabbit atria

Rabbit atria suspended in a 60 ml. organ-bath responded to adrenaline (0.1 to 5 μg), noradrenaline (0.02 to 2 μg) or isoprenaline (0.002 to 0.02 μg) with an increase in the rate and the amplitude of contractions. Within these ranges of doses, the responses of the atria to the catechol amines were easily reversed by washing out the organ-bath and the responses to each dose changed very little over a period of 2 to 3 hr.

In the presence of phenoxybenzamine, the effects of adrenaline and noradrenaline were increased, but the responses to isoprenaline remained unchanged. The lowest concentration of phenoxybenzamine that increased the response to adrenaline was 0.01 μg/ml. Progressively larger responses to adrenaline were obtained in the presence of 0.1 and 0.3 μg/ml. of phenoxybenzamine. Maximal enhancement of the response to adrenaline occurred with 1 μg/ml. of phenoxybenzamine. However, this concentration of phenoxybenzamine increased the rate and amplitude of contractions of the atria. It seemed simpler to use a lower concentration of phenoxybenzamine, which did not change the rate of beating of the atria, than to attempt to evaluate the degree of potentiation of adrenaline or noradrenaline in the presence of a steadily increasing rate of beating. Part of an experiment is illustrated in Fig. 1, from which it is clear that phenoxybenzamine (0.5 μg/ml.) increased the response to noradrenaline, while not changing the control rate and amplitude of contractions; the response to isoprenaline was not affected by phenoxybenzamine. In the experiment illustrated in Fig. 1, phenoxybenzamine clearly enhanced the ability of noradrenaline to increase the amplitude of contractions as well as potentiating its effects upon rate of beating. This effect was seen in most experiments,

but no attempt was made to measure it, because the amplitude of contractions, measured with a spring lever, depended upon the rate of beating of the atria. Cocaine (0.5 μg/ml.) or guanethidine (0.4 μg/ml.) acted like phenoxybenzamine. Each drug potentiated noradrenaline more than adrenaline and left the responses to isoprenaline unaffected. The potentiations of adrenaline and noradrenaline by phenoxybenzamine were apparently irreversible since they remained unchanged for

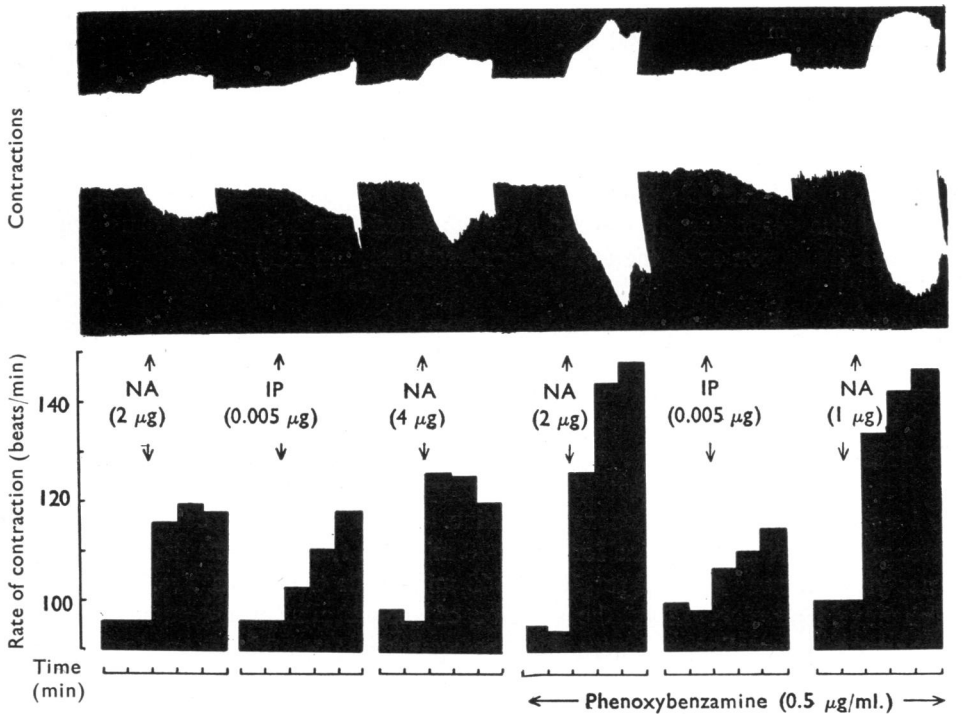

Fig. 1. The effects of noradrenaline (NA) and isoprenaline (IP) on the amplitude and rate of contractions of rabbit atria suspended in a 60 ml. organ-bath. In the presence of phenoxybenzamine (0.5 μg/ml.), the response to 1 μg of noradrenaline is considerably greater than the control response to 4 μg. The response to isoprenaline is not appreciably altered by phenoxybenzamine.

several hours after the phenoxybenzamine was washed out. The degree of potentiation produced by guanethidine could be reduced by repeated washing, but the responses to adrenaline and noradrenaline did not return completely to their control levels after washing out the guanethidine. The effect of cocaine was readily reversible. Maximal potentiation by phenoxybenzamine was seen about 30 min after its addition to the organ-bath ; guanethidine and cocaine produced maximal potentiation within the 12 min interval that was left between the additions of one of the catechol amines to the organ-bath. The results are summarized in Table 1. The mean values for potentiation indicate how much greater was the amount of catechol amine required to produce the same response in the absence of the potentiating drug.

TABLE 1

SELECTIVE POTENTIATION OF NORADRENALINE AND ADRENALINE ACTING ON RABBIT ATRIA BY PHENOXYBENZAMINE, GUANETHIDINE AND COCAINE

Values are means with standard errors of the degree of potentiation, which was calculated from the horizontal displacement of the dose/response line in the presence of the potentiating drug. Numbers of experiments are in parentheses

	Noradrenaline	Adrenaline	Isoprenaline
Phenoxybenzamine (0·5 μg/ml.)	7·93±1·23 (7)	2·77±0·34 (7)	0·90±0·06 (7)
Guanethidine (0·4 μg/ml.)	7·06±1·89 (6)	2·29±0·34 (3)	1·05±0·14 (6)
Cocaine (0·5 μg/ml.)	5·33±0·60 (6)	2·46±0·22 (5)	0·82±0·11 (5)

Vane (1962) has suggested that many basic drugs may increase the actions of noradrenaline by combining with nonspecific acidic radicles in tissues. With this in mind, hexamethonium (5 μg/ml.) and gallamine (20 μg/ml.) were tested to see if they increased the responses of isolated atria to noradrenaline. Each drug slightly increased the responses of the atria to noradrenaline, but only by 1.5-times.

Rat uterus

Phenoxybenzamine (0.03 or 0.06 μg/ml.) did not affect the sensitivity of the rat uterus to noradrenaline or to adrenaline in four experiments in which the right and left horns of uteri from two rats were used. Responses were obtained to each of two dose levels of adrenaline or noradrenaline, and then phenoxybenzamine was added whenever the organ-bath fluid was replaced, so that the preparation was continuously exposed to phenoxybenzamine. The assay was continued in the presence of phenoxybenzamine for about 20 min. In two experiments, the addition of acetylcholine was stopped for 10 and 60 min, while the uterus was left exposed to phenoxybenzamine. Then the addition of acetylcholine was resumed and the responses to adrenaline and noradrenaline were again recorded. In none of the experiments was there any increase in the responses to noradrenaline or adrenaline. The higher concentration of phenoxybenzamine (0.06 μg/ml.) reduced the height of the contractions produced by acetylcholine. Neither cocaine (0.5 μg/ml. ; four experiments) nor guanethidine (0.4 μg/ml. ; two experiments) affected the sensitivity of the rat uterus to noradrenaline or adrenaline.

Rabbit duodenum

In concentrations as low as 1 ng/ml., phenoxybenzamine decreased the sensitivity of the duodenum to noradrenaline and adrenaline. Increasing the concentration of phenoxybenzamine to 1 μg/ml. made little difference to its effect; the responses to noradrenaline and adrenaline were reduced but not abolished. However, phenoxybenzamine (0.5 μg/ml.) increased the sensitivity of the duodenum to isoprenaline about three-times.

In concentrations of 1 μg/ml., allowed to act for 15 to 30 min, neither guanethidine nor cocaine affected the sensitivity of the duodenum to noradrenaline, adrenaline or isoprenaline. A higher concentration of cocaine (10 μg/ml.) reduced the tone and the spontaneous contractions of the duodenum, thus making unreliable any

estimate of a change in sensitivity to the catechol amines. However, this high concentration of cocaine appeared to increase slightly the sensitivity of the duodenum to noradrenaline, adrenaline and isoprenaline, but any increase in sensitivity was less than two-fold.

Almost all of the catechol amine content of the intestine of the ox, sheep and dog consists of dopamine (Schümann, 1960), and so it seemed possible that cocaine might enhance the actions on the duodenum of dopamine more than of noradrenaline or adrenaline. However, cocaine (1 μg/ml.) did not increase the action of dopamine on the duodenum but, instead, blocked it, which suggests that dopamine does not act directly upon adrenaline receptors. It is interesting that Bejrablaya, Burn & Walker (1958) found that dopamine acted indirectly on the heart.

DISCUSSION

The results show that cocaine, guanethidine and phenoxybenzamine potentiate noradrenaline more than adrenaline in their actions on rabbit atria and leave the response to isoprenaline unchanged. It is believed that, in the heart, noradrenaline, adrenaline and isoprenaline each acts directly on the same receptors and, therefore, whatever may be the mechanism of their selective potentiation, it is unlikely that any alteration of the receptor sites is involved.

Maxwell, Plummer, Daniel, Schneider & Povalski (1958) and Maxwell, Plummer, Povalski, Schneider & Coombs (1959) suggested that guanethidine, cocaine and methyl phenidate, a group of drugs with otherwise unrelated pharmacological properties, all altered or " deformed " the structure of the adrenaline receptor. The evidence was that these drugs restored the responses to noradrenaline and adrenaline in the presence of phentolamine and small doses of dibenamine (that is, during competitive blockade) but not during complete nonequilibrium blockade produced by larger doses of dibenamine.

Macmillan (1959) explained the potentiating action of cocaine by suggesting that it blocks the access of noradrenaline to storage sites in the tissue and thus diverts a greater proportion of the administered dose onto the receptors. Macmillan's hypothesis has been extended and modified by others (Muscholl, 1961 ; Furchgott & Kirpekar, 1963). Macmillan's suggestion also explains why Maxwell *et al.* (1958, 1959) saw reversal only during competitive and not during nonequilibrium blockade. If the storage sites have a higher affinity for noradrenaline than for adrenaline, and no affinity at all for isoprenaline, the suggestion would also explain why noradrenaline is potentiated more than adrenaline, and why isoprenaline is not potentiated at all. Cocaine (Muscholl, 1961), phenoxybenzamine (Axelrod, Hertting & Potter, 1962) and guanethidine (Bhagat & Shideman, 1963) each blocks the uptake of noradrenaline into heart tissue, and each potentiates noradrenaline acting on isolated atria. Vane (1962) has suggested that many basic drugs can potentiate the actions of noradrenaline and adrenaline by blocking access to " non-specific take-up sites or sites of loss." However, these terms do not aptly describe the results with isolated atria. These sites seem to be specific, as they take up noradrenaline more than adrenaline and isoprenaline not at all, and this uptake is blocked much more

effectively by cocaine, guanethidine and phenoxybenzamine than by higher concentrations of hexamethonium or gallamine, which were taken as examples of the other basic drugs.

The conclusions drawn from experiments with isolated atria could not be extrapolated to other tissues, since the actions of noradrenaline or adrenaline on the rabbit intestine or rat uterus were not potentiated by phenoxybenzamine, guanethidine or cocaine. Rozkowski & Koelle (1960) found that cocaine did not increase the actions of noradrenaline, adrenaline or isoprenaline on isolated rabbit intestine or on guinea-pig tracheal chain. Phenoxybenzamine increased the action of isoprenaline on rabbit intestine, in agreement with Furchgott (1960), but not on rat uterus. Holzbauer & Vogt (1955) described a large (up to twenty-fold) increase in the actions of adrenaline and isoprenaline on the rat uterus by phenoxybenzamine. However, they stated that the degree of potentiation by phenoxybenzamine depended upon the initial sensitivity of the uterus, and that little potentiation was seen if the uterus was initially very sensitive to adrenaline or isoprenaline. Perhaps this explains the discrepancy between the results described here and those of Holzbauer & Vogt (1955). The uteri used in my experiments were sensitive to adrenaline in a concentration of 0.05 ng/ml., which was the threshold concentration mentioned by Holzbauer & Vogt. It is possible that it is the content of noradrenaline in the tissue stores that determines the sensitivity of the uterus.

If it is true that phenoxybenzamine, guanethidine and cocaine potentiate noradrenaline and adrenaline by blocking their uptake into storage sites in the tissue, it becomes necessary to find an explanation for the different results with the heart, intestine and uterus. Perhaps the storage sites have different properties in each tissue. Schümann (1960) has found that about 98% of the catechol amines stored in the intestine is dopamine. This raises the possibility that noradrenaline is less avidly taken up by the storage sites in intestine than in other tissues. This, if it is so, could explain the lack of potentiation of noradrenaline or adrenaline on rabbit intestine.

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