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TISSUE AMINE LEVELS AND SYMPATHETIC BLOCKADE AFTER GUANETHIDINE AND BRETYLIUM

BY

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A single dose of guanethidine produces a substantial, long-lasting depletion of tissue catecholamines in the rat, whereas a similar dose of bretylium has no effect. Both drugs produce block of the eserine-induced sympathetic pressor effect. Block by guanethidine is induced more rapidly than is amine depletion. When amine depletion is maximal, a noradrenaline infusion is capable of restoring the response to eserine, but no restoration of the response to eserine occurs after noradrenaline infusion into bretylium-treated rats. Catecholamine levels in isolated tissues are not reduced when complete block of sympathetic nerve stimulation has been produced by guanethidine. It is suggested that guanethidine possesses a primary bretylium-like, and a secondary reserpine-like, blocking action. Guanethidine produces a transient lowering of intestinal 5-hydroxytryptamine, and this coincides with increased intestinal motility.

The mechanism of the anti-hypertensive action of guanethidine and bretylium appears to be dissimilar from that of other agents in clinical use. Both drugs impair postganglionic adrenergic transmission and only possess a slight and transient ganglion blocking activity. Neither antagonizes the actions of adrenaline or noradrenaline. It has been suggested that they prevent the liberation of adrenergic transmitter from the nerve endings (Maxwell, Mull & Plummer, 1959; Maxwell, Plummer, Povalski & Schneider, 1960; Maxwell, Plummer, Schneider, Povalski & Daniel, 1960; Boura & Green, 1959). Guanethidine, in contrast to bretylium, depletes tissue catecholamine levels, and the suggestion has been made that this ability is related to the sympathetic blockade (Cass, Kuntzman & Brodie, 1960; Shepherd & Zimmermann, 1959; Burn, 1961). It was decided, therefore, to test this latter hypothesis by comparing the rate of onset and duration of sympathetic block with the rate and duration of amine depletion. This was done using a single dose of guanethidine in intact animals and some isolated tissues. Comparison was made with bretylium in some of the experiments. 5-Hydroxytryptamine levels in the tissues of animals after treatment with these agents were also measured.

METHODS

Male rats, Wistar strain, 160 to 210 g body weight, were given a single dose, by subcutaneous injection, of 15 mg/kg of guanethidine or bretylium, or of 0.9% sodium chloride solution. Six rats from each group were killed at various time intervals after injection. Heart, spleen

and small intestine were removed for assay of catecholamine or 5-hydroxytryptamine levels. Tissues were rinsed with water, blotted on filter paper, weighed and stored at −10° C until required.

Extraction and assay. Tissues were homogenized in 0.01 N hydrochloric acid and extracted with sodium chloride and acid-saturated butanol, which excludes dopamine. The amines in an aliquot of the butanol were returned to a small volume of 0.01 N hydrochloric acid by shaking with heptane. The acid layer was divided into two portions for the spectrophotofluorometric assay of catecholamine and 5-hydroxytryptamine respectively. The catecholamines (adrenaline plus noradrenaline) were estimated in terms of noradrenaline in heart, spleen and small intestine. The amines were oxidized at *p*H 5 with iodine, the fluorescence then being developed in an alkaline ascorbate solution. This was measured at an activating wavelength of 400 mμ and an emission wavelength of 515 mμ. 5-Hydroxytryptamine fluorescence was read at *p*H 2 using an activating wavelength of 300 mμ and an emission wavelength of 540 mμ. This is essentially the method of Shore & Olin (1958) with the addition that 5-hydroxytryptamine is measured in the same sample (J. A. R. Mead, unpublished). The only further modification was that, after oxidation, the samples were subjected to ultra-violet irradiation for 7 min in order to speed up the development of maximum fluorescence and read immediately thereafter.

Isolated tissues. Pieces of rabbit intestine stimulated through the periarterial sympathetic nerve (Finkleman, 1930), and guinea-pig vas deferens stimulated through the hypogastric nerve (Boyd, Chang & Rand, 1960; Huković, 1960), were used, bathed in McEwen's solution (1956) aerated with oxygen plus 5% carbon dioxide. A section of rabbit ileum was set up in an isolated organ bath (vol. 100 ml.) at 37° C and the nerve stimulated for 30 sec every 4 min at a frequency of 50/sec from a constant voltage output. Alternate sections of ileum were set up under comparable conditions and either guanethidine added to the bath and stimulation continued until complete block was obtained or stimulation without the drug continued for the same length of time. Vasa deferentia from guinea-pigs were set up in a similar manner, one horn being stimulated in the presence of guanethidine until complete block was obtained, the other being stimulated for the same length of time in the absence of the drug. Tissues were removed from the bath, dried on filter paper, weighed and stored at −10° C until extracted for assay.

Blood pressure recording. Rats were anaesthetized with urethane (150 mg/100 g) intraperitoneally. The blood pressure was recorded from the carotid artery using a Condon manometer. Both femoral veins were cannulated with polythene tubing for the injection of drugs. The degree of sympathetic function present was measured by the pressor response to an intravenous injection of 20 μg of eserine (Varagić, 1955; Lešić & Varagić, 1961). Infusions of noradrenaline or dopamine were made at a rate of 0.05 ml./min over 20 min, through one femoral vein cannula, the other being used for the injection of eserine. The guanethidine and bretylium were injected subcutaneously.

Drugs. Bretylium was used as the tosylate, doses being in terms of the base. Doses of guanethidine were in terms of the sulphate. Eserine salicylate (with sodium metabisulphite 0.1 mg/ml. as preservative) was used; dose was of eserine base.

RESULTS

Amine depletion. Guanethidine greatly reduced the noradrenaline levels in spleen, heart and intestine. The effect was apparent at 1 hr, and reached a maximum of 80 to 90% at 6 to 18 hr. Some recovery had occurred by 48 hr (Fig. 1). Bretylium had no effect on noradrenaline levels in heart or spleen, but there was a rise in intestinal noradrenaline from 2 to 6 hr after injection and this had returned to normal by 18 hr.

The 5-hydroxytryptamine content of the spleen was not altered by either agent. No 5-hydroxytryptamine was detectable in the rat hearts. There was a transient

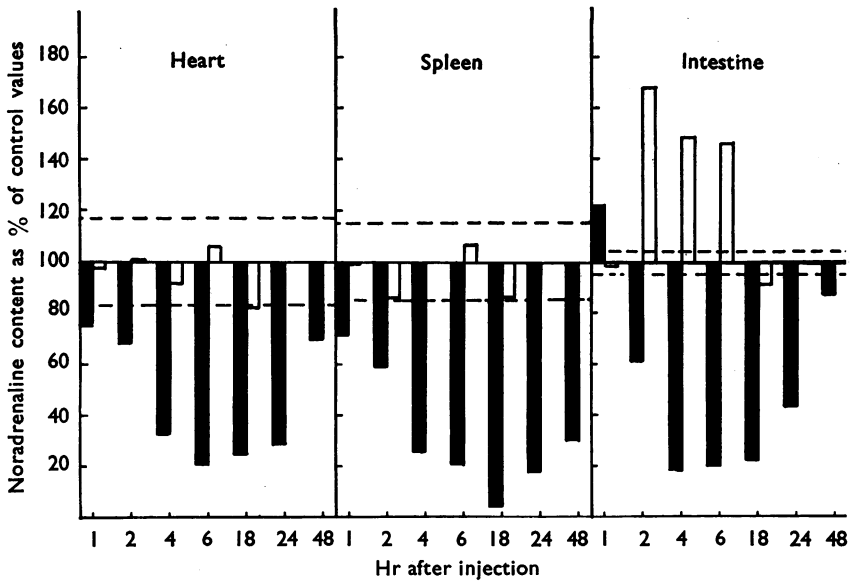

Fig. 1. Noradrenaline content of rat tissues after the subcutaneous injection of guanethidine 15 mg/kg (black columns) or bretylium 15 mg/kg (open columns). The dotted lines represent the standard error of the control levels.

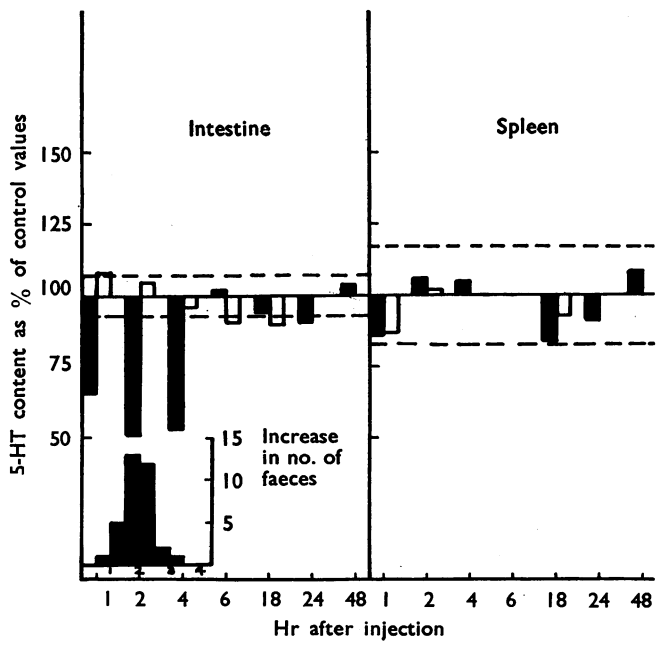

Fig. 2. 5-Hydroxytryptamine content of rat tissues after the subcutaneous injection of guanethidine 15 mg/kg (black columns) or bretylium 15 mg/kg (open columns). The dotted lines represent the standard error of the control levels. Inset: number of faeces passed by groups of rats after guanethidine, expressed as increase over the numbers passed by control groups. Abscissae: time in hr after injection.

depletion of intestinal 5-hydroxytryptamine after guanethidine which was maximal after 2 hr, returning to normal by 6 hr after injection. Diarrhoea was always a marked feature of the guanethidine-treated animals. When the number of faeces passed was counted at 30 min intervals, there was a large increase in the guanethidine-treated group compared with the other two groups (Fig. 2).

Sympathetic blockade. The pressor response to eserine was elicited repeatedly at 30 min intervals over a period of at least 6 hr. The height of the response was 26.3 ± 1.2 mm of mercury. It was confirmed that this response was present in rats adrenalectomized 2 weeks previously, but was eliminated in pithed rats. The injection of guanethidine resulted in an immediate fall of blood pressure, and subsequent injections of eserine were virtually without effect (Fig. 3). Some 6 hr

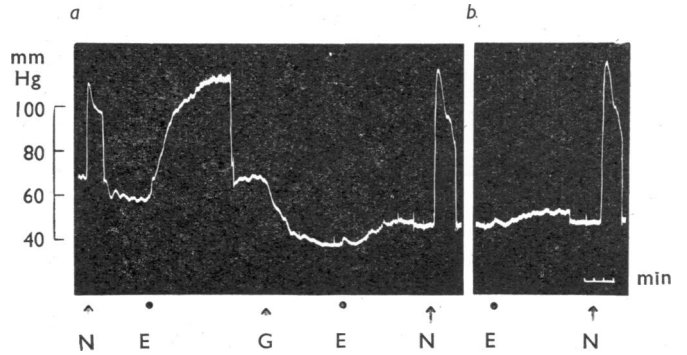

Fig. 3. Rat anaesthetized with urethane. Recording of blood pressure from carotid artery. The effect of guanethidine on the response to eserine. N=0.5 μg noradrenaline injected intravenously, E=20 μg eserine injected intravenously, G=guanethidine 15 mg/kg injected subcutaneously. Panel (*b*) shows the response 6 hr after G. Drum stopped after each response. E administered at 30 min intervals.

later the eserine response had not returned. When rats were treated 24 hr previously with guanethidine and the blood pressure recorded, the response was 10.8 ± 2.05 mm of mercury, that is, a 60% inhibition of the response to eserine. By 48 hr, however, the response was 24.6 ± 0.96 mm of mercury, which was not significantly different from the control response (Fig. 4). The pressor response to tetramethylammonium

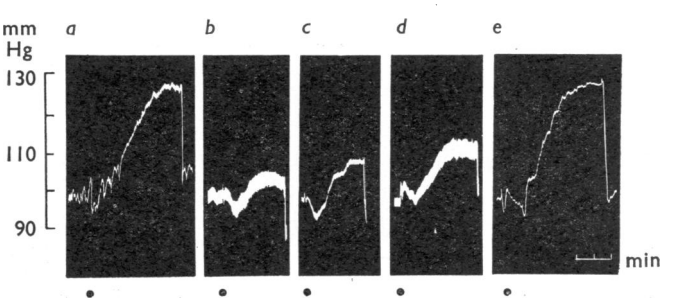

Fig. 4. Rat anaesthetized with urethane. Blood pressure recorded from the carotid artery. Typical responses to 20 μg eserine intravenously at the black dots, at various times after guanethidine 15 mg/kg subcutaneously. (*a*) Normal response, (*b*), (*c*), (*d*) and (*e*) respectively from rats injected with guanethidine 6, 18, 24 and 48 hr previously.

was not reduced by guanethidine and was sometimes potentiated. It was abolished by hexamethonium. Bretylium also caused a reduction of the response to eserine, but the onset was much slower and the degree of block was less.

Dopamine in doses up to 0.4 mg was ineffective in restoring the eserine response after blockade by guanethidine. However, 10 μg of noradrenaline sometimes increased the response, but its effectiveness was dependent on the time of infusion after the administration of guanethidine. Fig. 5 shows that noradrenaline when

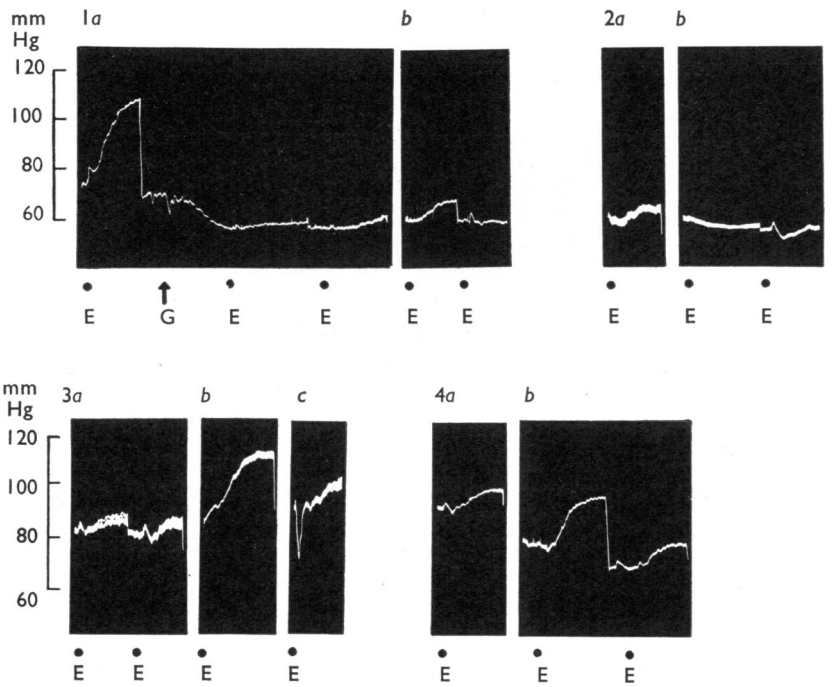

Fig. 5. Rat anaesthetized with urethane. Blood pressure recorded from the carotid artery. The effect of noradrenaline infusion on the response to eserine after guanethidine (G) 15 mg/kg subcutaneously. At E intravenous injection of 20 μg eserine. Between (*a*) and (*b*) 10 μg noradrenaline infused over 20 min. 1: 1·5 hr after G; 2: 3·5 hr after G; 3: 6 hr after G; and 4: 24 hr after G. Each number represents a different rat. 3(*c*) shows the response to eserine 30 min after 3(*b*).

infused 1 to 4 hr after guanethidine had little or no effect on the eserine response, but from 6 to 24 hr the response was restored. The infusion of noradrenaline had no effect on the response to eserine up to 18 hr after bretylium treatment.

Isolated tissues. Assay of segments of rabbit ileum or guinea-pig vas deferens in which guanethidine, in doses of 1 to 8 μg/ml., had produced complete block of the response to sympathetic nerve stimulation showed no depletion of noradrenaline content (Table 1): in fact, there was a slight increase. This increase was just significant ($P=0.05$) for the rabbit ileum.

TABLE 1

THE EFFECT OF GUANETHIDINE ON THE NORADRENALINE CONTENT OF RABBIT ILEUM (μG/G) AND GUINEA-PIG VAS DEFERENS (μG/HORN)

	Guanethidine	
Tissue	Absent	Present
Rabbit ileum	0·29±0·016	0·33±0·011
Guinea-pig vas deferens	1·17	1·46
	1·17	1·50
	1·47	1·72
	1·46	1·73

DISCUSSION

In the experiments described, an attempt has been made to test the hypothesis that the sympathetic block produced by guanethidine is related to a lack of tissue noradrenaline. Noradrenaline levels have been measured in heart, spleen and intestine and a marked and long-lasting depletion demonstrated. This is in agreement with the findings in rabbit and cat reported by Cass *et al.* (1960), and extends the observations reported for rats by Shepherd & Zimmermann (1959). These authors found a 50% depletion in heart and spleen 2 hr after 15 mg/kg guanethidine, but no effect after 24 hr. However, in their experiments the injection was made intraperitoneally and this may account for the much shorter duration of action. While these gross tissue levels are not necessarily a measure of changes in local concentration at the nerve terminals, a correlation between rate of measurable depletion and onset of sympathetic blockade would be a good indication that such a relationship existed.

Sympathetic function has been assessed by measuring the pressor response to a standard intravenous dose of eserine; this response has been shown by Lešić & Varagić (1961) to be mediated centrally. We have demonstrated that the response is reproducible over long periods, and that variation between animals is small. The response is unaltered by adrenalectomy and is absent in pithed rats. This confirms the findings of Lešić & Varagić and their conclusion that adrenal medullary discharge plays no part in the response. Guanethidine abolishes this response immediately, and in the anaesthetized rat the effect lasts at least up to 6 hr. There is evidence to suggest that the anaesthetic prolongs the primary blocking action of guanethidine (perhaps by slowing the rate of detoxication). In anaesthetized rats injected with guanethidine, there is no return of the eserine response over 6 hr. In rats given the drug less than 2 hr before the anaesthetic there is no response to eserine, but when guanethidine is administered more than 2 hr before the anaesthetic there is often a small response to the eserine and this does not increase over the period of the experiment. Since there is complete block of the response when the drug is given within 2 hr of the anaesthetic, comparison of the tissue levels measured in non-anaesthetized animals with the responses of the blood pressure during this period is valid.

Since Maxwell, Plummer, Schneider, Povalski & Daniel (1960) have demonstrated a short-lasting ganglion blocking action after guanethidine in the cat, the action of a ganglion stimulant drug, tetramethylammonium, has been tested before and during the first hour after guanethidine. Tetramethylammonium induced a rise of

blood pressure which was either unaffected or increased after guanethidine but which was abolished by hexamethonium. Since tetramethylammonium continued to stimulate the release of amines from the adrenal medulla in the presence of guanethidine, ganglion transmission appeared to be unaffected. The potentiation of the released amines probably compensates for the loss of activity at the post-ganglionic sympathetic sites.

It is clear, therefore, that the rate of onset of sympathetic block does not parallel the rate of depletion of peripheral noradrenaline. As previously discussed, this does not exclude a local but unmeasurable loss at the nerve ending. However, the results obtained using isolated tissues and those obtained by infusing noradrenaline into guanethidine-treated animals also indicate that there is no relationship between noradrenaline levels and the immediate blocking action of guanethidine. When block of the response to sympathetic nerve stimulation in the isolated tissues used was induced by guanethidine, there was no measurable loss of noradrenaline ; indeed, there was an increase, which for the rabbit ileum was statistically just significant. If the gross measurable changes are a slower reflection of small local changes it is unlikely that a measurable loss would be found within the time (8 to 44 min) that the tissues were exposed to guanethidine. If, in fact, the block were dependent on loss of transmitter, it is even more unlikely that a measurable increase would occur. It is interesting to note that, in the rat, intestinal noradrenaline was slightly increased at 1 hr after guanethidine injection, although by 2 hr depletion had occurred. Likewise after bretylium there was an increase in intestinal noradrenaline and this was sustained over 6 hr, bretylium having no depleting action. Schümann (1959) has shown in ox, sheep and dog that the principal catecholamine in the intestine is dopamine (95 to 100%). If this also applies to rabbit and rat it might provide an explanation for the increase in noradrenaline level seen in intestine. If the postulate that guanethidine and bretylium act by preventing the release or distribution of noradrenaline at the nerve ending is true, then treatment with these agents might be expected to result in a slight accumulation of noradrenaline. In tissues where noradrenaline or adrenaline are the principal catecholamines this increase might be masked, but in the intestine, where the proportion is normally very small, the increase might be revealed. Experiments are in progress to investigate this possibility.

Burn & Rand (1958, 1960) have shown that the response to certain sympathomimetic amines and to sympathetic stimulation, absent in the reserpine-treated animal, is restored by the infusion of noradrenaline or some of its precursors. Therefore the effect of infusions of dopamine or noradrenaline on the response to eserine after guanethidine or bretylium was investigated. The dose used was one which Burn & Rand had shown to be effective in restoring the response to tyramine in a pithed reserpine-treated rat. In our experiments it had little or no effect during the first few hours of guanethidine block, but, during the period when tissue noradrenaline levels were less than 25% of normal, noradrenaline infusion usually restored the eserine response to within limits normal for untreated rats. After bretylium, noradrenaline never increased the eserine response even in higher dosage. This again suggests that the primary block by guanethidine is unrelated to noradrenaline levels and that it may be similar to bretylium. It does, however, suggest

that the maintenance of the block may be due to a lack of noradrenaline. Burn (1961), in a review of adrenergic nerve function explaining the action of reserpine, bretylium and guanethidine, suggested that the hypotensive action of guanethidine was reserpine-like, that is, due to lack of noradrenaline. He also pointed out that guanethidine has a bretylium-like property. The evidence presented here suggests that this is the primary action of guanethidine and the reserpine-like action is secondary. The finding that dopamine, in doses 4 times those found by Burn & Rand to be effective in reserpine-treated rats, was ineffective in the guanethidine-treated rats is surprising. It may be due to the fact that our experiments were carried out in anaesthetized animals, whereas the results of these authors were obtained in pithed animals. Alternatively the use of eserine might have reduced the activity of dopamine dehydrogenase. Experiments are in progress to clarify these points.

One of the most common side-effects reported after clinical use of guanethidine is diarrhoea (Leishman, Matthews & Smith, 1959 ; Dollery, Emslie-Smith & Milne, 1960). Since 5-hydroxytryptamine has been implicated in the production of peristalsis both experimentally in animals (Bülbring & Lin, 1958) and in man (Lembeck, 1958), it was decided to investigate the action of both agents on 5-hydroxytryptamine levels. There was no alteration of spleen 5-hydroxytryptamine, but in the intestine guanethidine induced a 50% depletion after 2 hr. No 5-hydroxytryptamine has been detected in rat heart, but in rabbit and cat hearts no depletion of 5-hydroxytryptamine occurs after guanethidine (Cass *et al.*, 1960, and unpublished observations). The maximal depletion in the intestine coincided with the peak of peristaltic activity as measured by the number of faeces passed in unit time. Bretylium neither altered intestinal 5-hydroxytryptamine nor increased intestinal motility. This suggests a relationship between the liberated 5-hydroxytryptamine and the intestinal motility. It is not clear in this instance whether the 5-hydroxytryptamine released into the lumen of the intestine stimulates peristalsis as shown by Bülbring & Lin, or whether the guanethidine has a direct effect and the 5-hydroxytryptamine is released as a consequence of the increased activity. Reserpine induces marked diarrhoea, and it is one of the most common features of the carcinoid syndrome in which large amounts of 5-hydroxytryptamine are secreted from tumour tissue. These facts would indicate the first possibility as being the most likely. Since, however, the loss of 5-hydroxytryptamine after guanethidine seems to be specific to the intestine, the second possibility cannot be ruled out.

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