THE EFFECTS OF GUANETHIDINE ON THE NORADRENALINE CONTENT OF THE HYPOTHALAMUS IN THE CAT AND RAT

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Single injections of guanethidine 15 mg./kg. i.p. did not lower the hypothalamic noradrenaline in cats, but, daily injections of 15 mg./kg. s.c. over a 7 day period consistently produced a decrease in the hypothalamic noradrenaline. Attempts to lower the hypothalamic noradrenaline in rats after single injections or daily injections of guanethidine were unsuccessful.

Guanethidine, 2-(octahydro-1-azocinyl) ethyl guanidine sulphate, is a hypotensive drug, which produces a depletion in the noradrenaline content in the peripheral tissues (Butterfield and Richardson, 1961; Cass, Kuntzman and Brodie, 1961; Cass and Spriggs, 1961; Sanan and Vogt, 1962). Although the levels of noradrenaline are not reduced to the extent necessary to block impulse transmission, this depletion possibly contributes to its hypotensive action.

Conflicting reports occur about whether guanethidine causes a depletion of noradrenaline in the brain. Cass and Spriggs (1961) were unable to demonstrate a change in the noradrenaline content of the cat or rabbit brain and attributed this lack of an effect to the inability of the highly ionised molecule to penetrate the blood-brain barrier. Significant decreases have been reported recently in the rat brain within 1–3 hr. after acute administration of guanethidine (Pfeifer, Vizi and Satory, 1962). Sanan and Vogt (1962) in some but not all experiments observed decreases in the hypothalamic noradrenaline of the cat and rabbit. This effect was inconsistent from animal to animal and especially from colony to colony. This suggested a possible reflex stimulation of the sympathetic centres and not a direct effect.

Further experiments are reported here on the effect of acute and chronic administration of guanethidine in cats and rats on the content of noradrenaline in the hypothalamus.

Methods

Male or female cats, bred in Babraham and weighing 1·7–2·8 kg. were bled under chloroform anaesthesia. The tissues were removed quickly, weighed, and placed into acid-ethanol (0·1 ml. conc. HCl to 100 ml. ethanol) chilled in a dry ice-acetone mixture until extracted. Female albino rats, 175–225 g., were decapitated; the hypothalamus removed, weighed and placed into chilled acid ethanol. The portion of hypothalamus removed excluded the corpora mammillaria and optic tracts.

The tissues were extracted and purified using procedures previously described (Vogt, 1952, 1953, 1954). Noradrenaline was determined by

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the bioassay method using the pithed rat blood pressure (Muscholl and Vogt, 1957.) Recoveries were checked in some experiments by adding 0·1 μg. noradrenaline to about 0·1 g. of cerebellar tissue in which the concentration of noradrenaline is known to be less than 0·1 μg./g. (Vogt, 1954). There was no pooling of samples, the tissue of each animal being extracted singly.

Guanethidine sulphate was injected as a 1–2 per cent solution in 0·9 per cent saline. Doses are expressed in terms of the weight of the salt. In acute experiments in both cats and rats, injections were given intraperitoneally. For chronic administration the solution was "sterilised" by bringing it to the boil. In chronic experiments in cats the "sterilised" solution was given subcutaneously, and in rats it was injected subcutaneously.

RESULTS

At various times up to 72 hr. after a single injection of guanethidine, 15 mg./kg., in cats, the hypothalamic content of noradrenaline was not lowered but in the superior cervical ganglia the content of noradrenaline was significantly lowered (Table I). Yet examination of the ranges of

TABLE I

NORADRENALINE CONTENT IN HYPOTHALAMUS AND SUPERIOR CERVICAL GANGLIA OF CATS AT VARIOUS TIMES AFTER GUANETHIDINE, 15 MG./KG., I.P. NORADRENALINE IN μG./G. FRESH TISSUE, MEAN ± S.E. OF THE MEAN CORRECTED FROM RECOVERIES OF 50–70 PER CENT. RANGE IN BRACKETS. *SIGNIFICANT DECREASES ($P<0·05$) TESTED BY "t" TEST ANALYSIS

Time interval (hr.)	No. of animals	Hypothalamus	Superior cervical ganglia
0	5	2·3 ± 0·19 (1·8–2·9)	10·6 ± 1·5 (6·9–12·2)
4	2	1·8 ± 0·24 (1·6–2·1)	5·2 ± 0·7* (5·1–5·3)
16	5	1·6 ± 0·30 (0·71–2·7)	4·0 ± 1·2* (1·2–7·0)
24	2	2·6 ± 0·94 (1·7–3·6)	4·0 ± 1·2* (2·9–5·1)
72	3	2·4 ± 0·14 (2·2–2·8)	7·2 ± 1·4* (4·3–9·2)
7 day chronic 15 mg./kg. s.c.	5	0·85 ± 0·12* (0·60–1·1)	4·0 ± 0·54* (2·0–4·9)

the results for the hypothalamus indicated individual variations and single figures which were low. This was especially true 16 hr. after guanethidine when the range was 0·71–2·7 μg./g. To rule out the possibility that larger doses may have depleted more effectively, a cat was given 25 mg./kg., but 16 hr. later the noradrenaline content of the hypothalamus of this animal was 2·5 μg./g., near the upper limit of the normal range. In contrast, daily injections over a 7 day period consistently produced a significant decrease in the hypothalamic noradrenaline (Table I).

Overt signs in these cats indicated depression of the sympathetic nervous system. After a single injection there was a partial relaxation of the nictitating membrane, slight miosis, some closure of the palpebral fissure, and some reduction in motor activity. No phase of early excitement was evident in these cats. All cats had diarrhoea. With the chronic treatment

similar signs of partially relaxed membrane, slight miosis, and a generally quiet demeanour were present. In addition, there was some anorexia but little loss of weight. After 3 days, two of the cats showed marked restlessness and resistance to petting; on the last day, they appeared apprehensive and, if excited by the presence of strangers, signs of adrenal medullary stimulation (mydriasis, contraction of the nictitating membrane, and panting) became evident.

In one experiment with rats, a dose of 5 mg./kg. was given and the hypothalamus removed after a time interval of 3 hr. This procedure was the same as that of Pfeifer, Vizi and Satory (1962). In an additional group of rats an injection of 15 mg./kg. was given. There was no effect on the hypothalamic noradrenaline after a single dose of guanethidine and in other experiments there was no effect after chronic administration (Table II). Yet in the group receiving 5 mg./kg. once, there was a wider range (0·56–1·43 μg./g.) of noradrenaline contents than in control rats, as noted in cats. The only signs observed in rats was diarrhoea.

TABLE II

NORADRENALINE CONTENT OF THE HYPOTHALAMUS IN RATS AFTER SINGLE AND DAILY SUBCUTANEOUS INJECTIONS OF GUANETHIDINE. CONTROLS INJECTED WITH 0·9 PER CENT NACL SOLUTION. NORADRENALINE IN μG./G. WET TISSUE, MEAN ± S.E. OF THE MEAN. RANGE IN BRACKETS

Dose of guanethidine (mg./kg.)	3 hr. after single injection		24 hr. after the last of 7 daily injections	
	μg./g.	No. of rats	μg./g.	No. of rats
0	1·2 ± 0·11 (0·91–1·46)	5	1·4	1
5	1·1 ± 0·12 (0·56–1·40)	6		
15	1·2 ± 0·08 (1·0–1·40)	4	1·4 ± 0·04 (1·3–1·5)	5

The failure to obtain consistent effects on the noradrenaline content of the hypothalamus of the rat with sufficient guanethidine to cause a clear depletion in the cat, prompted experiments with another drug, β-tetrahydronaphthylamine, which, by stimulating sympathetic centres, regularly lowers the hypothalamic noradrenaline in the cat (Vogt, 1954).

Four rats were given β-tetrahydronaphthylamine, 30 mg./kg., subcutaneously and killed 4 hr. later. The signs of sympathetic stimulation seen were exophthalmos, piloerection, restlessness, rise in temperature, and increased rate of respiration. The hypothalamic content of noradrenaline was decreased to 0·88 ± 0·08 μg./g., but this difference was not statistically significant from the central means of 1·2 ± 0·11. The result suggests that stimulation of the sympathetic centres by β-tetrahydronaphthylamine does not affect the brain noradrenaline of the rat as readily as that of the cat.

DISCUSSION

Sanan and Vogt (1962) suggested a reflex stimulation of the sympathetic centres to explain the occasional decrease of the hypothalamic noradrenaline after a single dose of guanethidine. The consistent fall in the

hypothalamic content of noradrenaline after giving repeated daily doses of guanethidine is compatible with this idea. The effect was seen in the cat which reacts to stimulation of the sympathetic centres by β-tetrahydronaphthylamine with a fall in brain noradrenaline (Vogt, 1954). This effect of guanethidine was absent in the rat in which the hypothalamic noradrenaline was also more resistant to depletion by β-tetrahydronaphthylamine.

Another possibility is that after prolonged administration, sufficient quantities of guanethidine may pass the blood-brain barrier to exert a direct effect in depleting noradrenaline from the hypothalamus. Kaneko, McCubbin and Page (1962) conducted experiments in dogs to test the central effect of guanethidine. In their experiments, systemic injections of guanethidine were ineffective, but injections given intraventricularly or intracisternally caused a central inhibition of vasomotor tone. These workers suggested that prolonged clinical use of guanethidine could result in a situation where enough of the drug passed the blood-brain barrier to exert an effect on the cardiovascular system.

The third possibility is that the action of guanethidine is, indeed, indirect, but due to enhanced central sympathetic activity elicited as a result of lack of a feed-back mechanism. The centres might be overactive in response to the inhibition by guanethidine of the peripheral adrenergic neurones.

The report by Pfeifer, and others (1962) that brain noradrenaline was depleted after one dose of guanethidine in the rat was not confirmed. The difference in the results can hardly be attributed to the fact that I was measuring the noradrenaline in the hypothalamus, rather than in the whole brain. Other drugs which have been tested produce similar losses of noradrenaline in the midbrain and in the hypothalamus (Vogt, 1954), and these two regions together contain a large fraction of the noradrenaline of the whole brain. There is, however, the possibility that different strains of rats react differently. In addition, different methods of extraction were used.

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