THE EFFECTS OF THIOURACIL, ALLOXAN AND NICOTINE ON THE SUPRARENAL GLANDS

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THE suprarenal medulla of most animals contains both adrenaline and noradrenaline. Although it is fairly certain that adrenaline is formed from noradrenaline by methylation, the exact way in which noradrenaline is produced in the body is still an open question. Recently, with the aid of paper chromatography, Goodall[1] showed that hydroxytyramine, in addition to adrenaline and noradrenaline, may be present in sheep suprarenal glands, and that dihydroxyphenylalanine may be found in the glands of thyroidectomised sheep. These two substances may therefore be precursors of noradrenaline. We have now examined suprarenal extracts of rabbits to which thiouracil has been given, to try to determine such precursors. We have also tested suprarenal extracts of rabbits made diabetic with alloxan, since Hökfelt[2] showed that the suprarenal glands of diabetic rats contained a very low content of adrenaline and noradrenaline. If suprarenal exhaustion occurs in this condition, it might be possible to detect precursors of adrenaline in the suprarenal gland.

Injections of nicotine over long periods (13 to 17 months) may produce chromaffin adenoma in the suprarenals of young rats and suprarenal hyperplasia in adults.[3] In rabbits, continued administration of nicotine (97 to 124 days) has been shown to decrease and then to increase the adrenaline content of the suprarenal glands.[4] Since this treatment may also be associated with changes in the concentrations of precursors of adrenaline, we have given daily doses of nicotine to rabbits, rats and guinea-pigs. After a suitable interval of time, we have examined extracts of their suprarenal glands. In some experiments, daily doses of methionine (a methyl donor) have also been given. It was expected that the methionine-treated animals would possess a more rapid rate of methylation in the suprarenals than that found in the other groups.

METHODS

Rabbits, guinea-pigs and rats were killed by a blow on the head and their suprarenal glands were removed as soon as possible. After removal of the capsule, the glands were weighed and ground with sand and 1 to 5 ml. of 0·01 N hydrochloric acid/g. The extracts were centrifuged and the clear supernatant liquids were assayed for their adrenaline and noradrenaline contents by paper chromatography and biological assay (see Shepherd and West[5]). Careful examination of the paper chromatograms was carried out in every case to detect hydroxytyramine and dihydroxyphenylalanine.

Subcutaneous injections of thiouracil (in slightly alkaline solution)

were given daily (except Sundays) to 6 rabbits. Other rabbits received alloxan (100 mg./kg. intravenously) after food had been withdrawn for 48 hours. For the nicotine experiments, groups of 2 rabbits, rats or guinea-pigs were injected daily (except Sundays) with varying doses of nicotine acid tartrate. After a certain number of subcutaneous injections, they were killed either 6 hours or 7 days after the last injection. In other experiments, nicotine and methionine were given to groups of rabbits and rats. Solutions of *l*-adrenaline, *l*-noradrenaline bitartrate, hydroxytyramine hydrochloride, and *dl*-dihydroxyphenylalanine in 0·01 N hydrochloric acid were used as controls.

RESULTS

Effect of thiouracil in the rabbit. After 15 doses of thiouracil (50 mg./kg.), the mean value of extracts from 2 rabbits was 505 μg. of adrenaline/g., noradrenaline being absent. No major change has

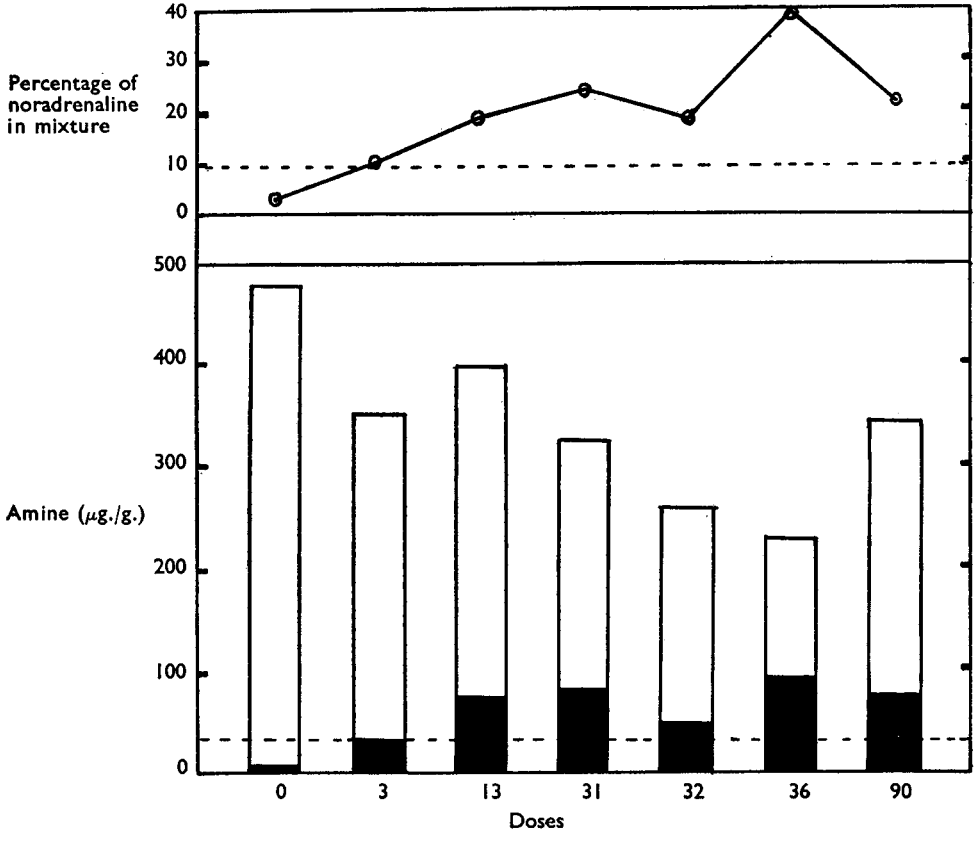


FIG. 1. The effect of daily doses of nicotine acid tartrate (16 mg./kg.) on the amine content (lower histogram) and the relative noradrenaline content (upper graph) of the suprarenal glands of rabbits. Plain areas, adrenaline; shaded areas, noradrenaline. The broken lines represent the maximal absolute and relative noradrenaline contents of the glands from 56 control rabbits.

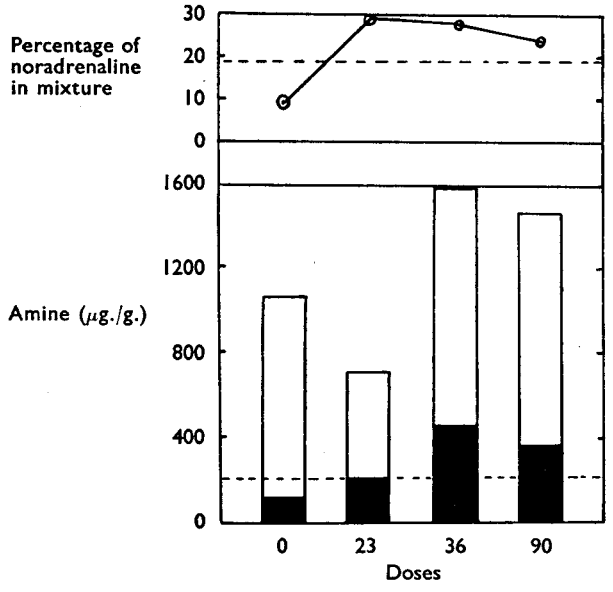


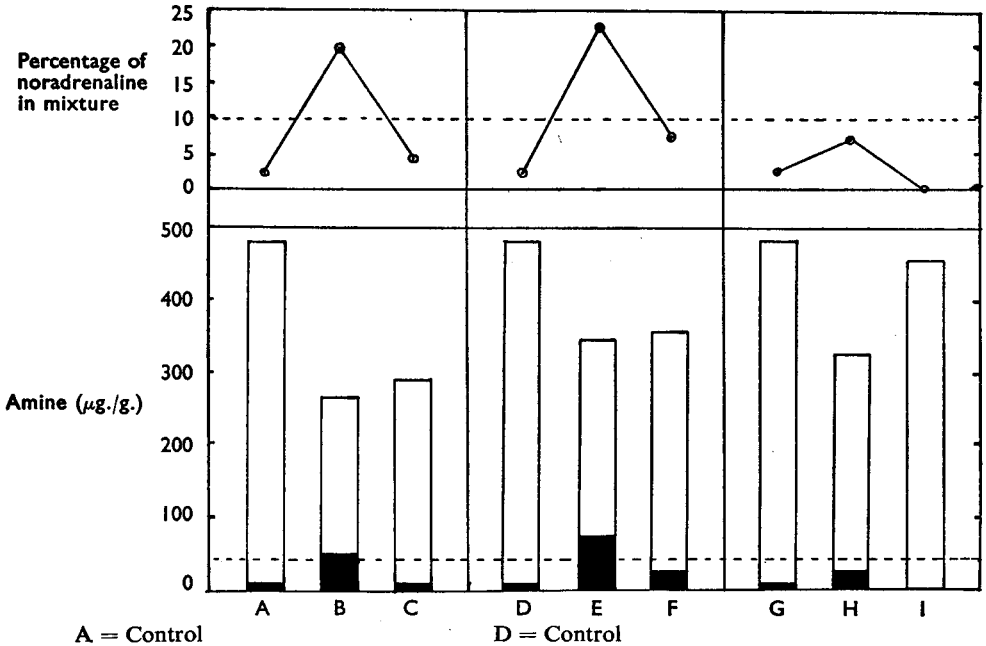
FIG. 2. The effect of daily doses of nicotine acid tartrate (2 mg./kg.) on the amine content (lower histogram) and the relative noradrenaline content (upper graph) of the suprarenal glands of rats. Plain areas, adrenaline; shaded areas, noradrenaline. The broken lines represent the maximal absolute and relative noradrenaline contents of the glands from 24 control rats.

occurred therefore in the amine content of their suprarenal glands, since the mean values of extracts from 56 adult control animals are 470 μg. of adrenaline and 10 μg. of noradrenaline/g. (Shepherd and West[5]).

2 other rabbits received 40 doses of thiouracil and lost weight (3·2 to 2·8 kg.). Their suprarenal glands appeared to be partly exhausted, values of 150 μg. of adrenaline and 2 μg. of noradrenaline/g. being recorded. Another 2 rabbits received 40 doses of thiouracil followed by insulin (4 I.U./kg. subcutaneously). 3 hours after the insulin, they were killed. Apart from further suprarenal exhaustion (mean value of 50 μg. of adrenaline/g.), no precursors of adrenaline were detected.

Effect of alloxan in the rabbit. When diabetes was confirmed in these animals, they were killed (about 10 days after the dose of alloxan). Suprarenal exhaustion had occurred (mean value of 61 μg. of adrenaline/g.) but again no precursors of adrenaline were detected.

Continued administration of nicotine. Rabbits received daily subcutaneous doses of 16 mg./kg. and the results are shown in Figure 1. Since the maximal noradrenaline content of the suprarenal glands from 56 control rabbits is 35 μg./g. (shown by the dotted line), it is clear that even after 13 doses of nicotine the noradrenaline values are raised, whilst total activity figures are somewhat decreased. Despite this increase in the relative noradrenaline content of the suprarenal gland, hydroxytyramine or dihydroxyphenylalanine were not identified. The rabbits



A = Control
B = 32 doses killed at 6 hours
C = 32 doses killed at 7 days
D = Control
E = 90 doses killed at 6 hours
F = 90 doses killed at 7 days
G = Control
H = 90 doses and 90 doses of methionine killed at 6 hours
I = 90 doses and 90 doses of methionine killed at 7 days

FIG. 3. The effect of daily doses of nicotine acid tartrate (16 mg./kg.) on the amine content (lower histogram) and the relative noradrenaline content (upper graph) of the suprarenal glands of rabbits. Plain areas, adrenaline; shaded areas, noradrenaline. The broken lines represent the maximal absolute and relative noradrenaline contents of the glands from 56 control rabbits. Also shown is the effect of 90 doses of nicotine and methionine (24 mg./kg.) on the amine content of the suprarenal glands of rabbits. Some animals killed 6 hours after the last injection, others left for 7 days. Note the protective action of methionine.

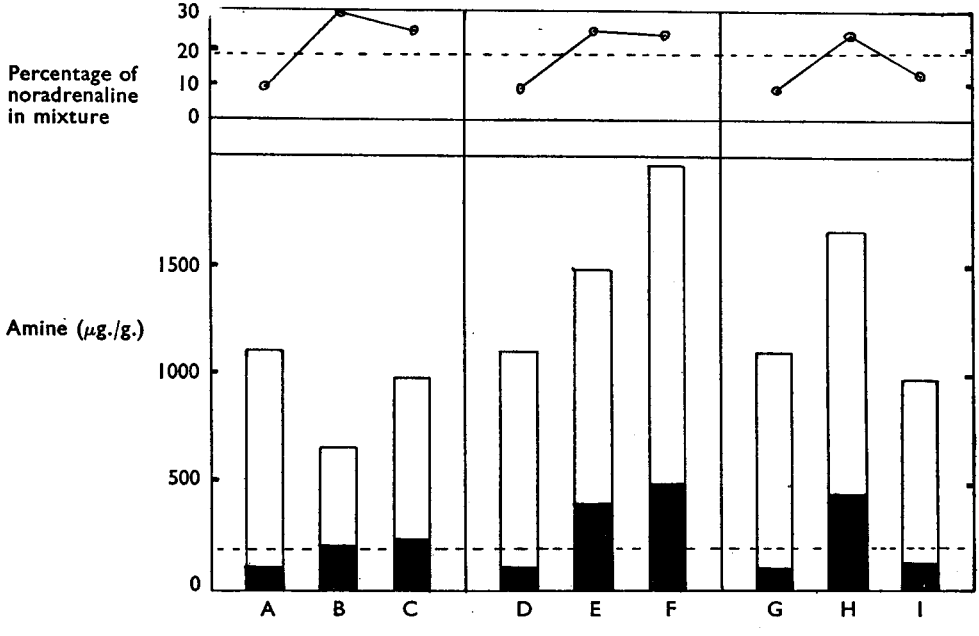
lost weight during the first 2 or 3 weeks of treatment, but then steadily gained. After each nicotine dose, respiration was stimulated and there was loss of power in the hind-limbs.

Rats received daily subcutaneous doses of 2 mg./kg. and the results are shown in Figure 2. With 23 doses there is a decrease in total activity, but after further doses there is an increase. As in the rabbit experiments, the increase in relative noradrenaline content was not accompanied by the appearance of detectable amounts of other precursors. Stimulation of the central nervous system (tail erection, circular movement sometimes backwards) was usually noted after each nicotine dose. Respiration was depressed, probably due to depression of the respiratory centre.

Guinea-pigs received 36 daily subcutaneous doses of 8 mg./kg. When killed, their suprarenal glands contained only adrenaline, and total activity values were low (mean of 50 μg./g.) compared with the mean

value of 60 control guinea-pigs (122·5 μg. adrenaline and 2·5 μg. noradrenaline/g. tissue). General depression was noted in these animals after each nicotine dose.

Continued administration of nicotine and methionine. In Figure 3 are shown the effects of 32 and 90 doses of nicotine (16 mg./kg.) and of 90 doses of nicotine and methionine (24 mg./kg.) on the amine content of the suprarenal glands of rabbits. The total activity was decreased in all three experiments, but whereas the relative noradrenaline content was increased following nicotine dosage it was not significantly increased when nicotine and methionine were given together. Therefore the depressant effect of nicotine on the rate of methylation in the rabbit may be reduced by the administration of excess of methionine (that is, by giving a methyl donor). In the rat, on the other hand (Fig. 4), it is clear that the rate of methylation is decreased even in the presence of excess of methionine (3 mg./kg.).



A = Control
B = 23 doses killed at 6 hours
C = 23 doses killed at 7 days
D = Control
E = 90 doses killed at 6 hours
F = 90 doses killed at 7 days
G = Control
H = 90 doses and 90 doses of methionine killed at 6 hours
I = 90 doses and 90 doses of methionine killed at 7 days

FIG. 4. The effect of daily doses of nicotine acid tartrate (2 mg./kg.) on the amine content (lower histogram) and the relative noradrenaline content (upper graph) of the suprarenal glands of rats. Plain areas, adrenaline; shaded areas, noradrenaline. The broken lines represent the maximal absolute and relative noradrenaline contents of the glands from 24 control rats. Also shown is the effect of 90 doses of nicotine and methionine (3 mg./kg) on the amine content of the suprarenal glands of rats. Some animals killed 6 hours after the last injection, others left for 7 days. Note the rapid return to normal levels in the presence of excess of methionine.

Also shown in Figures 3 and 4 are the results of leaving some treated animals for 7 days after the last injection before testing their suprarenal extracts. In rabbits receiving only nicotine, recovery of the rate of methylation occurred during this period although total activity remained low. In rabbits receiving both nicotine and methionine, recovery of both the rate of methylation and the total activity rapidly took place. In rats receiving 23 doses of nicotine, recovery was slow in this period, little change being recorded in the relative noradrenaline content of the glands. After 90 doses, however, there was a large increase in the total activity with little or no change in the raised relative noradrenaline content. In rats receiving both nicotine and methionine, recovery of both total activity and rate of methylation was almost complete in this period. Methionine therefore speeded up the recovery phase in the glands of both rabbits and rats.

Histological examination. Examination of frozen sections stained with hæmatoxylin and eosin, Heidenhain's iron hæmatoxylin, Mallory's stain, or acid fuschin and methyl green, indicated that only minor changes had occurred within the suprarenals. Reduction in the amount of stainable material in the medulla was particularly noticeable in the nicotine-treated animals. No neoplastic tissue was visible in the medulla.

Discussion

These experiments were designed to detect precursors of adrenaline by influencing the normal production of adrenaline in the suprarenal medulla. However, only noradrenaline has been found and then not in every extract. Since the limit for the detection of hydroxytyramine and dihydroxyphenylalanine by paper chromatography is 5 μg./g., less than 1 per cent. of the total catechols could be present as these substances.

In rabbits receiving thiouracil for 40 days, the total activity of the glands was reduced to one-third of the control value without affecting the relative noradrenaline content. Subsequent treatment with insulin produced further suprarenal exhaustion with little or no change in the relative amounts of the amines present. Therefore, the normal production of adrenaline in the medulla has not been altered by this thiouracil treatment. A similar finding was obtained with diabetic rabbits so that hypothyroidism and diabetes apparently do not influence the rate of methylation in the suprarenal medulla of rabbits.

The influence of nicotine is of considerable importance because of the great prevalence of smoking. The general response of the organism to nicotine is in part due to adrenaline, the output of which is increased soon after the injection and remains at a high level for several minutes. Since tumours of the suprarenal gland have been reported in the literature following nicotine administration over long periods, it was of interest to see the effects in the rabbit whose gland contains almost entirely adrenaline. Besides possible tumour formation, there seemed every possibility that more than one precursor of adrenaline would be identified. However, 90 doses of nicotine did not produce drastic changes. A steady decrease in total catechol activity was noted in rabbits, whereas the

decrease was followed by an increase in rats; the relative noradrenaline content increased in both rats and rabbits but no other precursor appeared. Methionine had little effect on the action of nicotine in rats but did allow of faster methylation in rabbits. When doses were stopped and the animals were left for 7 days, the methionine-treated animals recovered quicker than the controls, i.e. methionine accelerated the rate of methylation in animals whose suprarenals had been partly exhausted or stimulated. Previous workers have already shown that the methyl group of methionine given to rats may be found as the $N-CH_3$ groups of adrenaline isolated from the adrenal gland (Keller, Boissonas and du Vigneaud[6]).

Summary

1. If thiouracil or alloxan is given to rabbits, suprarenal exhaustion occurs but the relative noradrenaline content of the gland is not raised.

2. The administration of nicotine to rats and rabbits for periods up to 90 days results in a lowered rate of methylation of noradrenaline. Simultaneous dosage of methionine provides some protection. In the recovery phase following the injections, the methionine-treated animals possess a more rapid rate of methylation in the suprarenals than that of the control animals.

3. In no experiments were dihydroxyphenylalanine or hydroxytyramine detected in adrenal extracts by the chromatographic method.

I wish to record my thanks to Dr. D. M. Shepherd of this Department for most of the chromatographic studies, and to Dr. A. T. McQueen of the Physiology Department, University College, Dundee, for supplying the glands of alloxan-treated rabbits. Professor A. Haddow of the Chester Beatty Cancer Research Institute supplied the reference to work on experimental adrenal medullary tumours.

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