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THE EFFECT OF PRENYLAMINE ON THE METABOLISM OF CATECHOL AMINES AND 5-HYDROXYTRYPTAMINE IN BRAIN AND ADRENAL MEDULLA

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In 1960, Schöne & Lindner reported that the administration of prenylamine (Segontin, Hoechst) to rats reduced the concentration of noradrenaline in the brain; if the dose was high enough (100 mg/kg), the brain 5-hydroxytryptamine level was also lowered and some sedation was observed. The same authors showed in 1962 that adrenal medullary granules lose their catechol amines in the presence of 10^{-4} M-prenylamine. This was confirmed by several workers (Carlsson, Hillarp & Waldeck, 1963; Euler, Stjärne & Lishajko, 1964), who also showed that smaller concentrations prevented the uptake of amines and decreased their spontaneous release into the medium.

The fact that the effects of such a fairly simple derivative of amphetamine (prenylamine is *N*-(3,3-diphenylpropyl)-α-methylphenethylamine) showed striking resemblance to those of reserpine prompted a further investigation of the mode of action of prenylamine.

METHODS

Groups of male albino rats weighing 220 to 350 g were given subcutaneous injections of prenylamine gluconate, 50 to 100 mg/kg, and the animals were killed at intervals thereafter. Male albino rabbits (body weight 2.1 to 2.8 kg) were injected subcutaneously either with prenylamine gluconate (100 mg/kg) or with reserpine (Serpasil, Ciba; 2 mg/kg).

Estimation of dopamine (3,4-*dihydroxyphenylethylamine*) *and noradrenaline.* The tissues were weighed, homogenized in hydrochloric acid, and the proteins precipitated with perchloric acid as described by Dagirmanjian, Laverty, Mantegazzini, Sharman & Vogt (1963). The neutralized supernatant fluid was applied to a column of the resin Dowex 50-X 8 (Bertler, Carlsson, Rosengren & Waldeck, 1958). The noradrenaline, together with any adrenaline present, was eluted with 10 ml. of 0.4 N-hydrochloric acid and the dopamine with 8 ml. of 2 N-hydrochloric acid. The noradrenaline estimation was done by oxidation with ferricyanide (Euler & Lishajko, 1961) to form a fluorescent trihydroxyindole; details are found in Sharman, Vanov & Vogt (1962). Dopamine was estimated by fluorimetry after condensation of its acetylated derivative with ethylenediamine (Laverty & Sharman, 1965a). The estimation of both amines was carried out with a Locarte fluorimeter.

Recoveries of dopamine. During experiments in the spring recoveries were erratic, sometimes as low as about 20%. This was traced to the effect of bright daylight and disappeared when the columns were protected from light by a shade. The mean recovery before this effect was known was 61%, and this figure has been used to correct all results obtained in daylight. In the shade, recoveries rose to a mean of 74%, and this figure was used for correction in the appropriate experiments.

Recoveries of noradrenaline. The mean recovery of noradrenaline was 85%.

Estimation of homovanillic acid. The method of extraction was that described by Sharman (1963a). The deproteinized tissue homogenate was saturated with solid sodium chloride, extracted with ethylacetate

and the concentrated extract applied to alkali-washed Whatman No. 50 paper and chromatographed in a mixture of benzene, propionic acid and water (100 : 70 : 5). The chromatograms were run for 15 hr at room temperature. The appropriate strips were placed in test tubes and eluted with 3 ml. of water, the tubes being shaken at intervals and the papers allowed to soak for at least 1 hr.

Two methods were used for the fluorimetric estimation of homovanillic acid in the eluates and gave the same results. The first was a reaction with ferric chloride and sodium hydroxide (Sharman, 1963a), and the second employed ferricyanide and ammonia (Andén, Roos & Werdinius, 1963) to form fluorescent compounds. In both instances, the fluorescence was read in a Locarte fluorimeter using as primary filter two thicknesses of Chance OX7, and as secondary filters Corning 3389 (standard) and Corning 5113 (half standard thickness).

For the estimation of the very low values of homovanillic acid in the brain stem of the rat it was necessary to obtain values for internal standards, tissue blanks and recoveries. The procedure was as follows. The brain stem of four rats (about 1.3 g of tissue) was extracted, chromatographed and eluted as described, and 1 ml. of the eluate used to estimate the fluorescence developed by the endogenous homovanillic acid (reading 1); another 1 ml. of eluate, to which 0.2 μg of homovanillic acid was added, was used as one of three " internal standards " (reading 2). In addition, 2.6 g of brain tissue from which brain stem had been removed (mainly cerebellum and cerebral hemispheres) was homogenized and divided into two equal portions. One of these was worked up like the brain stem; 1 ml. of eluate gave the " tissue blank " (reading 3), and another 1 ml., to which 0.2 μg of homovanillic acid was added, a second " internal standard " (reading 4). The other portion of homogenate was used to measure recovery; 2 μg homovanillic acid was added, the tissue processed as before, and the fluorescence measured in 1 ml. of eluate in order to estimate the recovery (reading 5). A second 1 ml. of eluate, to which a further 0.2 μg homovanillic acid was added, yielded a third " internal standard " (reading 6). Also required was a paper blank (7), obtained by eluting a strip of paper to which no sample had been applied.

Reading 1 minus reading 7 gave the fluorescence attributable to endogenous homovanillic acid; the mean of differences 2−1, 4−3 and 6−5 represented the equivalent of fluorescence developed by 0.2 μg of standard in presence of brain tissue, and from these two figures the unknown amount was calculated. The difference 3−7 was always smaller than the difference 1−7, indicating that no or very little endogenous homovanillic acid was present outside the brain stem. Recoveries of homovanillic acid ranged from 44 to 60%, and figures were corrected for the mean of 50%.

Estimation of 5-hydroxyindolyl compounds. Basic and acidic compounds, corresponding mainly to 5-hydroxytryptamine and 5-hydroxyindolylacetic acid, were always analysed separately (Ashcroft & Sharman, 1962), the former by extracting the deproteinized tissue extract at *p*H 10 with *n*-butanol (Bogdanski, Pletscher, Brodie & Udenfriend, 1956), the latter by extracting with ether in the presence of hydrochloric acid (*p*H 1 to 2) (Udenfriend, Titus & Weissbach, 1955). An Aminco-Bowman spectrophotofluorimeter was used for the estimations.

Mean recoveries were 46% for 5-hydroxytryptamine and 40% for 5-hydroxyindolylacetic acid. As for the other substances, the results were corrected for the mean, not for the individual recoveries.

Dissections. For each dopamine estimation, a single rat brain was used, from which cerebellum, olfactory bulbs, hypothalamus and as much cortical tissue as possible had been removed. This " brain stem " extended caudally as far as the obex and weighed approximately 1 g. In the rabbit, both caudate nuclei were homogenized and one-quarter of the homogenate was taken for the estimation.

Noradrenaline was estimated in the hypothalamus of single rats; the optic nerves were dissected away, and the tissue weighed about 30 mg.

For the estimation of homovanillic acid the " anterior brainstem " of four rats was pooled. This was obtained by making a transverse section through the brain at the level of the infundibulum, discarding the caudal part of the brain and removing olfactory lobes and as much cortex as possible from the anterior part. In this way, the corpus striatum was the main component of the portion used. Each block of tissue weighed about 0.35 g. In the rabbit, three-quarters of the homogenate of one pair of caudate nuclei was used for each estimation.

The 5-hydroxyindoles were extracted from the whole brain of single rats after removal of cerebellum and olfactory lobes.

Prenylamine was used as the gluconate. The 5% solution was diluted four times with isotonic glucose for subcutaneous injection into rats, and twice for injection into rabbits. The undiluted solution is too irritant to inject.

Perfusion of the adrenal glands. Dog isolated adrenal glands were perfused by gravity at 35° C with Locke solution gassed with a stream of 5% carbon dioxide and 95% oxygen. Details of the dissection are published elsewhere (Vogt, 1965). Difficulties were encountered by cloudiness forming when the 5% prenylamine gluconate was diluted with Locke solution; this was prevented by diluting with bicarbonate-free Locke solution, saturating the mixture with 5% carbon dioxide and 95% oxygen, and adding the bicarbonate last. Solutions up to 10^{-4} (1.9×10^{-4} M) prepared in this way remained clear also when warmed to perfusion temperature in the jacketed reservoir used to deliver the perfusion fluid.

The effluent was collected for 2-min periods into ice-cooled measuring cylinders, and 0.1 ml. of N-hydrochloric acid was added to each 10 ml. of fluid collected. Extraction of the catechol amines, their paper chromatographic separation and bioassay have been described (Vogt, 1952; Muscholl & Vogt, 1964).

RESULTS

The dopamine, noradrenaline and homovanillic acid concentrations in the brain of normal rats and the effects produced by subcutaneous injection of prenylamine (50 or 100 mg/kg) are shown in Table 1 and Fig. 1.

At 1 hr after a dose of 50 mg/kg of prenylamine the concentration of dopamine in the brain stem had decreased. A somewhat greater fall was seen at 2, 4 and 24 hr, but values had returned to control level at 72 hr. By increasing the dose to 100 mg/kg a larger decrease in the amine concentration was obtained. The noradrenaline concentration in the hypothalamus followed the same pattern as that of dopamine although the decrease was less pronounced. This, however, may be due to an artefact. It will be seen in Table 1 that the

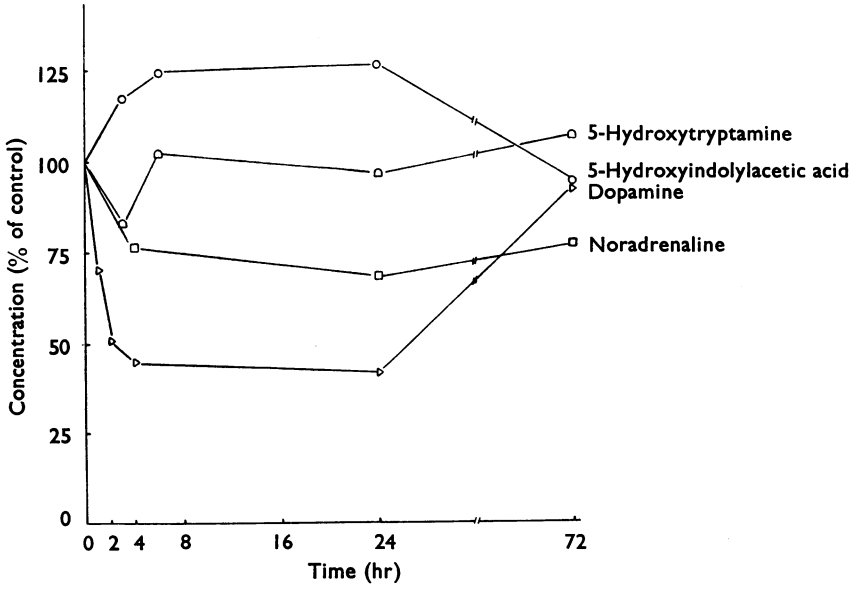

Fig. 1. The concentration of noradrenaline, dopamine, 5-hydroxytryptamine and 5-hydroxyindolylacetic acid in the brain of rats examined at various intervals after a single subcutaneous injection of prenylamine (50 mg/kg when catechol amines, 100 mg/kg when indoles were estimated). Abscissa: time in hours. Ordinate: percentage of control concentration.

TABLE 1

NORADRENALINE, DOPAMINE AND HOMOVANILLIC ACID IN RAT BRAIN AT VARIOUS INTERVALS AFTER SUBCUTANEOUS ADMINISTRATION OF A SINGLE DOSE OF PRENYLAMINE

Values are means and standard errors in μg/g of fresh tissue, and are corrected for recovery. * Each observation made on pools of four brains. The dose was 50 mg/kg except where † indicates that 100 mg/kg was used. No prenylamine was injected in the control experiments. Numbers of experiments are in parentheses

Duration of experiment (hr)	Brainstem dopamine (μg/g)	Hypothalamus noradrenaline (μg/g)	Brainstem homovanillic acid (μg/g)*
Control	1·16±0·06 (30)	3·61±0·18 (30)	0·14±0·01 (8)†
1	0·82±0·10 (6) $P<0·01$		
2	0·57±0·10 (6) $P<0·001$		0·12 (1)†
4	0·52±0·02 (7) $P<0·001$	2·75±0·32 (6) $P<0·025$	
24	0·48±0·05 (9) $P<0·001$	2·59±0·27 (10) $P<0·05$	
24	0·26±0·02 (10)† $P<0·001$	2·38±0·22 (12)† $P<0·001$	0·10±0·01 (5)†
72	1·06±0·06 (6) $P<0·2$	2·81±0·48 (8) $P<0·2$	

noradrenaline content of the hypothalamus of normal rats is stated to be 3.6 μg/g. Values obtained previously in this laboratory (Dagirmanjian, 1963) by bioassay and estimations carried out recently either by bioassay or by a fluorimetric method applied to the acetylated amine (J. E. McEwen & D. F. Sharman, personal communications) gave values which, after correction for recovery, ranged from 2.0 to 2.3 μg/g. There is not enough adrenaline present to account for such a large discrepancy. The rat hypothalamus either contains a substance which is adsorbed to the ion-exchange resin and produces a fluorescence on oxidation with ferricyanide but is not noradrenaline, or the "faded blanks" give an erroneously low value. If all figures of Table 1, column 3, are too high by the same amount, as is possible, the loss of amine produced by the drug will be partly obscured.

The homovanillic acid content of the brain was extremely low and remained unchanged after injections of prenylamine, though the dose used was always the higher one of 100 mg/kg. Control experiments were therefore carried out with reserpine which had been shown in the cat (Sharman, 1963b) and in the rabbit (Roos, Andén & Werdinius, 1964) to increase the homovanillic acid content of the caudate nucleus. As will be reported in detail elsewhere, no rise was found, so that it appears that increased turnover of dopamine in the rat does not, in contrast to other species, lead to an accumulation of homovanillic acid.

In order to obtain information on a species in which injection of reserpine raises the homovanillic acid content of the brain, a few experiments were carried out on rabbits. Table 2 shows that prenylamine, though causing a profound fall in the dopamine content, did not significantly raise the homovanillic acid of the caudate nucleus. In contrast, reserpine (2 mg/kg) caused the expected rise in homovanillic acid level.

The concentration of 5-hydroxytryptamine and of 5-hydroxyindol-3-ylacetic acid in the brain of normal rats, and the effect produced by a subcutaneous injection of prenylamine, 100 mg/kg, are shown in Table 3.

After 3 hr, there was a small but not statistically significant fall in the concentration of 5-hydroxytryptamine; at the same time, and also at 6 and 24 hr after the injection, the

TABLE 2

DOPAMINE AND HOMOVANILLIC ACID IN RABBIT CAUDATE NUCLEUS AFTER SUBCUTANEOUS ADMINISTRATION OF A SINGLE DOSE OF PRENYLAMINE (100 mg/kg) OR RESERPINE (2 mg/kg)

Values are means and standard errors in μg/g of fresh tissue, and are corrected for recovery. Numbers of experiments are in parentheses. * $P<0.001$

Drug	Duration of experiment (hr)	Dopamine (μg/g)	Homovanillic acid (μg/g)
None	0	9·8, 10·8 (2)	5·2±0·4 (7)
Prenylamine	4	1·4, 1·6 (2)	5·9±0·3 (5)
Prenylamine	24	—	5·2, 3·6 (2)
Reserpine	4	<0·5 (4)	9·1±0·4 (4)*

tissue content of 5-hydroxyindolylacetic acid, the acid metabolite of 5-hydroxytryptamine, was increased. At 72 hr the values were back to normal.

Effect of prenylamine on the dog isolated perfused adrenal gland. The glands of six dogs were perfused for a few minutes with concentrations of prenylamine ranging from 2.5×10^{-5} to 10^{-4} (4.8×10^{-5} M to 1.9×10^{-4} M). When the concentration was 10^{-4} or 5×10^{-5}, there was an increase in output of both medullary amines with noradrenaline predominating; the largest increases in output observed after 10^{-4} prenylamine were 1,150% (noradrenaline) and 367% (adrenaline). However, after washing out the prenylamine an injection of dimethylphenylpiperazinium iodide (20 μg) or an electrical stimulation of the splanchnic nerves had lost their normal effect, which is a large increase in release of medullary amines. When the concentration of prenylamine was reduced to 2.5×10^{-5}, release of amines was only little increased during perfusion and the effect of a subsequent injection of dimethylphenylpiperazinium was not suppressed but appeared to be smaller than usual.

TABLE 3

5-HYDROXYTRYPTAMINE AND 5-HYDROXYINDOLYLACETIC ACID IN RAT BRAIN AT VARIOUS INTERVALS AFTER SUBCUTANEOUS ADMINISTRATION OF PRENYLAMINE (100 mg/kg)

Values are means and standard errors in μg/g of fresh tissue, corrected for recovery, with numbers of experiments in parentheses. No prenylamine was injected in the control experiment. * Difference from controls statistically significant ($P<0.05$)

Duration of experiment (hr)	Brain 5-hydroxytryptamine (μg/g)	Brain 5-hydroxyindolylacetic acid (μg/g)
Control	0·58±0·03 (22)	0·42±0·02 (22)
3	0·48±0·04 (8)	0·49±0·03 (7)*
6	0·59±0·01 (8)	0·52±0·03 (8)*
24	0·56±0·03 (10)	0·53±0·05 (10)*
72	0·62±0·04 (6)	0·39±0·02 (6)

DISCUSSION

The experiments have shown that, in the rat, prenylamine lowers brain noradrenaline and dopamine levels and increases brain 5-hydroxyindolylacetic acid. There is thus an effect exerted on the metabolism of the three amines investigated, even if the moderate fall in 5-hydroxytryptamine concentration reported by Schöne & Lindner (1960) did not occur in our experiments; it was also not observed by Fresia, Consolo, Sioli & Valzelli (1963).

Whereas many drugs lower the noradrenaline content of the brain, only two groups are at present known to reduce simultaneously the concentration of both noradrenaline and

dopamine; one group is that of the reserpine-like substances, the other consists of the α-methyl-substituted aromatic aminoacids (α-methyl dihydroxyphenylalanine and α-methyl-metatyrosine).

In comparing the actions of prenylamine with that of reserpine-like drugs, the mildness of the pharmacological effects produced is striking. In the rat, there was no diarrhoea, narrowing of the palpebral fissure or change in temperature; some decrease in muscular tone was the only visible sign of drug action. In the rabbit, on the other hand, there was some miosis and ptosis in addition to reduction in muscular tone. Biochemically, after either drug there was a fall in noradrenaline and dopamine in the brain; and, in the rat brain, the homovanillic acid content remained the same but the 5-hydroxyindolylacetic acid was elevated.

In the rabbit, however, reserpine caused an accumulation of homovanillic acid in the caudate nucleus during the course of 4 hr, whereas prenylamine caused practically no change. Since, however, the fall in dopamine was also more drastic after reserpine than after prenylamine, the difference between the two responses may simply be one of degree and is in keeping with the mildness of all changes produced by prenylamine. Thus, the effect of prenylamine also differed from that of reserpine in that it hardly lowered the 5-hydroxytryptamine content of brain and depressed the other amines for a shorter period of time. There are, however, reserpine derivatives for which the same is true: dimethylaminobenzoylmethylreserpate (Su 5171), given to rabbits in small doses, lowers brain noradrenaline concentrations without seriously affecting brain 5-hydroxytryptamine; it does not sedate and its effects are not as prolonged as those of reserpine (Brodie, Finger, Orlans, Quinn & Sulser, 1960). It is, therefore, possible that prenylamine and Su 5171 are closely related in their mode of action, though information is lacking about tissue concentrations of dopamine, homovanillic acid, or 5-hydroxyindolylacetic acid after Su 5171 to support this suggestion.

There would also be grounds for classifying the action of prenylamine as akin to that of the α-methyl derivatives of aromatic amino acids. Hess, Connamacher, Ozaki & Udenfriend (1961) have shown that these substances lower guinea-pig brain dopamine and 5-hydroxytryptamine for a short, but noradrenaline for a very long, period. This was later explained by the fact that the amines formed by decarboxylation of the α-methyl amino acids act as " antimetabolites " of catechol amines (Creveling, Daly, Witkop & Udenfriend, 1962). Amphetamine also causes, in some species, a lowering of the noradrenaline (Sanan & Vogt, 1962) and of the dopamine content of brain (Laverty & Sharman, 1965b); competition for dopamine β-oxidase would explain the former, competition for dopamine binding sites the latter action. Since prenylamine is an amphetamine derivative, it is conceivable that its depleting actions on catechol amines, too, are due to competition at binding sites and enzymes. Competition at binding sites would be one explanation for the release of catechol amines seen in perfused adrenals. The fact, however, that there was irreversible damage to the release mechanism when the drug was withdrawn suggests that this effect may have been a nonspecific artefact. The perfusion fluid containing the drug was clear when saturated with 5% carbon dioxide, but it is possible that inside the tissue the *p*H was not low enough to prevent precipitation of the prenylamine and damage to cell membranes or capillaries. Such an interpretation would agree with the observation that, as less amine was released when the dose of prenylamine was reduced, there was also less interference with a sub-

sequent stimulation of the medulla by dimethylphenylpiperazinium. Furthermore, close-arterial injection by the method of Feldberg & Minz (1931) of prenylamine (1 mg) *in vivo* into the adrenal gland of an anaesthetized eviscerated cat did not produce a rise in blood pressure, indicative of release of catechol amines. There was only a fall which was much smaller than when the same dose was injected intravenously; amine release by a subsequent dose of dimethylphenylpiperazinium was not interfered with.

Reserpine does not release amines from the perfused adrenal medulla (Vogt, 1965). If the release caused by prenylamine is regarded as an artefact, the absence of action on the adrenal medulla would represent one more instance in which the two substances resemble each other.

It was surprising to find that the homovanillic acid content of the " anterior brainstem " was very low in the rat. Figures for the caudate nucleus of other species (Sharman, 1963a) range from an average of 3.5 μg/g in cat and sheep to somewhat higher figures in the rabbit and to about 10 μg/g in the dog. Caudate nucleus and putamen, the two nuclei which form the " striatum," should contain the same amount of homovanillic acid, so that the " anterior brainstem " of the rat, of which at least one-third is striatal tissue, should contain no less than 1 μg/g if the content were comparable to that of cat or sheep. In fact, after correction for recovery, the concentration was only one-tenth of that figure. Since this concentration is not raised after either prenylamine or reserpine, homovanillic acid either does not represent the main metabolite of dopamine in the rat, or is not held in the tissue but removed by the circulation as soon as it is formed.

SUMMARY

1. Single doses of prenylamine (50 to 100 mg/kg) injected subcutaneously into rats produced a fall in the dopamine content of the brainstem and of the noradrenaline of the hypothalamus.

2. The concentration of the dopamine metabolite homovanillic acid was very low in the brainstem of the rat, and it was increased neither by prenylamine nor by reserpine.

3. In rabbits, the dopamine content of the caudate nucleus fell to about 15% of its normal value after prenylamine (100 mg/kg), and to less than 5% after reserpine (2 mg/kg). Only after reserpine was there a significant rise of the homovanillic acid content of the caudate nucleus.

4. In rats, the 5-hydroxytryptamine of the brain was not changed after prenylamine, 100 mg/kg, but the 5-hydroxyindol-3-ylacetic acid was increased, thus indicating an ncreased turnover of 5-hydroxy tryptamine.

5. Prenylamine released medullary amines from the perfused adrenal gland of the dog; this release was followed by a block of the response to electrical stimulation of the splanchnic nerves or to an injection of dimethylphenylpiperazinium. This effect does not appear to occur *in vivo*.

6. In spite of the different chemical structure, it is likely that the mode of action of prenylamine is akin to that of the weaker analogues of reserpine.

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