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THE EFFECTS OF RESERPINE AND 6-HYDROXYDOPAMINE ON THE CONCENTRATIONS OF SOME ARYLALKYLAMINES IN RAT BRAIN

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1 The concentrations of *p*- and *m*-tyramine were measured in the caudate nucleus of the rat brain following subcutaneous injection of reserpine or intraventricular injection of 6-hydroxydopamine. β-Phenylethylamine was analysed in the hypothalamus after reserpine.

2 Endogenous levels of *p*-tyramine and *m*-tyramine in the caudate nucleus, and β-phenylethylamine in the hypothalamus were 8.02, 2.25 and 2.52 ng/g respectively.

3 Tyramine concentrations were reduced to less than 20% of control values one day after a reserpine injection of 1 or 10 mg/kg. A single dose of reserpine (0.4 mg/kg) significantly decreased the content of both tyramines in the caudate nucleus. The effects became apparent as early as 45 min after drug administration and persisted for at least 6 h in the case of *p*-tyramine and for at least 19 days in the case of *m*-tyramine.

4 The hypothalamic content of β-phenylethylamine was unaffected by reserpine.

5 Ten days after an intraventricular injection of 6-hydroxydopamine (250 μg), *p*- and *m*-tyramine concentrations in the caudate nucleus were significantly below control levels.

6 The results suggest that *p*- and *m*-tyramine may be stored by an intraneuronal reserpine-sensitive storage mechanism. Alternatively, the tyramines may replace some of the catecholamines from their storage granules and then be released as false transmitters by the nervous impulse. The observed changes in tyramine levels might also reflect the fact that these amines may be metabolically related to another amine which is stored in reserpine-sensitive granules.

Introduction

β-Phenylethylamine, *p*-tyramine, *m*-tyramine and tryptamine have been observed in small amounts in the mammalian brain (Saavedra & Axelrod, 1972; Durden, Philips & Boulton, 1973; Philips, Durden & Boulton, 1974a, b; Saavedra, 1974; Willner, LeFevre & Costa, 1974; Boulton, Juorio, Philips & Wu, 1975a; Philips, Davis, Durden & Boulton, 1975) and have been shown to be present in a synaptosomal fraction (Boulton & Baker, 1975). Brain concentrations of the neurotransmitters dopamine, noradrenaline and 5-hydroxytryptamine are known to be severely reduced by reserpine (Shore, Silver & Brodie, 1955; Holzbauer & Vogt, 1956; Bertler, 1961), presumably by impairment of a Mg^{2+}-adenosine triphosphate (ATP)-dependent storage mechanism (Carlsson, Hillarp & Waldeck, 1963). More recently it has been shown that reserpine also decreases the level of *p*-tyramine in the optic lobe of *Octopus* (Juorio & Philips, 1975). Administration of 6-hydroxydopamine intravenously to kittens or intraventricularly to mature rats depletes both noradrenaline and dopamine in the brain (Laverty, Sharman & Vogt, 1965; Ungerstedt, 1968; Uretsky & Iversen, 1970; Laverty & Taylor, 1970).

This study examines the effects of reserpine and 6-hydroxydopamine on the concentrations of β-phenylethylamine, *p*-tyramine and *m*-tyramine in the rat brain. For comparative purposes, the levels of dopamine, noradrenaline and 5-hydroxytryptamine were also measured. Some of the present results have been previously communicated to the Pharmacological Society (Boulton, Juorio, Philips & Wu, 1975b).

Methods

Male Wistar rats weighing 150–230 g were used. Crystalline reserpine (Sigma Chemical Co., St. Louis, Mo.), the amount depending on the dose to be injected, was dissolved in 0.1–0.2 ml of glacial acetic acid, diluted as required with isotonic glucose solution (at least 50-fold) and injected subcutaneously (2 ml/kg). Control animals were given a corresponding volume of the vehicle. 6-Hydroxydopamine hydrobromide (Sigma Chemical Co., St. Louis, Mo.) was dissolved in cold 0.9% w/v NaCl solution (saline) containing 10 mg/ml of ascorbic acid. Each

animal was anaesthetized with sodium pentobarbitone (20 mg/kg, i.p.), and 6-hydroxydopamine (250 μg of the base in 25 μl) was injected into the lateral ventricle of the brain (Noble, Wurtman & Axelrod, 1967). Control animals received an equivalent volume of the vehicle. After appropriate time intervals, the animals were decapitated and the brains quickly dissected into the following parts: the caudate nucleus (including some of the underlying putamen, mean weight 59 mg); the hypothalamus (the tissue below the thalamus and between the anterior and posterior commissura, mean weight 47 mg); the thalamus (mean weight 55 mg); and the 'brain stem' (including the posterior colliculi, the medulla oblongata and the pons to the obex, mean weight 236 mg). After dissection, the tissues were quickly frozen, weighed, and stored on dry ice until analysis (not more than one day).

Mass spectrometric analyses

β-Phenylethylamine, *p*-tyramine and *m*-tyramine were estimated as their dansyl derivatives by the mass spectrometric integrated ion current technique, with the corresponding deuterated compounds used as internal standards. The caudate nuclei from five animals were pooled for the analysis of *p*- and *m*-tyramine. After weighing, the tissues were homogenized in 5.5 ml of 0.1 N HCl containing 1 mg/ml of disodium edetate (EDTA) and 5 mg/ml of ascorbic acid, and 4 ml was removed for analysis; 1 ml was kept for the estimation of dopamine. The volume was adjusted to approximately 20 ml with 0.4 N perchloric acid, and deuterated *p*- and *m*-tyramine added (25 ng each of the free base). The purity of each deuterated compound was determined by analysing the dansyl derivatives of known amounts of the deuterated compound and the corresponding non-deuterated compound of established purity. By comparison of the areas enclosed by the respective integrated ion current curves (Durden *et al.*, 1973), the amount of deuterated amine actually present in the standard could be determined. A correction factor could be calculated and used to determine the actual amount of deuterated standard added to the tissue samples. An amine fraction was separated and the dansyl derivatives prepared as described previously (Philips *et al.*, 1974a, 1975). The dansylated isomers of *p*- and *m*-tyramine were purified by two successive unidimensional separations on 20 × 20 cm thin layers of silica gel (Brinkmann Instruments Ltd., Rexdale, Ont.) in the solvent systems chloroform : ethyl acetate, 4 : 1 (v/v), and benzene : triethylamine, 12 : 1 (v/v). The dansyl amines were eluted from the silica gel and analysed mass spectrometrically as described earlier (Philips *et al.*, 1974a; 1975).

To analyse β-phenylethylamine in the hypothalamus, tissue from five animals was pooled, weighed, then homogenized in 2 ml of saturated sodium carbonate containing deuterated phenylethylamine (25 ng of free base). The sample was frozen for 30–60 minutes. After thawing, the amines were extracted into two 1.5 ml portions of acetone and the dansyl derivatives prepared by adding 0.5 ml of dansyl chloride reagent (8 mg/ml in acetone). Following overnight reaction at room temperature, traces of sodium carbonate were precipitated by addition of 10 ml of acetone. The supernatant was transferred to a round bottomed flask and dried by rotary evaporation at 45°C. Dansyl derivates were eluted from the flask with ethyl acetate, reduced to a few drops under a stream of N_2, and transferred to a 20 × 20 cm thin layer of silica gel. The dansyl β-phenylethylamine zone was separated in the solvent system chloroform : ethyl acetate, 4 : 1 (v/v), and further purified on a second layer of silica gel by allowing the solvent system benzene : triethylamine, 8 : 1 (v/v) to rise to a height of 8 cm. The chromatogram was dried, then redeveloped in carbon tetrachloride : triethylamine, 5 : 1 (v/v) to a height of 18 cm. Dansyl β-phenylethylamine was eluted from the silica gel and analysed mass spectrometrically as previously described (Durden *et al.*, 1973).

Fluorimetric analyses

The catecholamines were estimated by the fluorimetric method proposed by Laverty & Sharman (1965) as described by Juorio (1971). Dopamine estimations were carried out in about one-fifth of the pooled caudate nucleus homogenate, while noradrenaline was estimated in the pooled thalamus obtained from two to five rats. The catecholamines were acetylated and the resulting tri-acetates separated by paper chromatography. The compounds were then eluted and condensed with 1,2-diaminoethane, and the fluorescent products extracted into isobutanol and estimated. 5-Hydroxytryptamine was extracted into butanol from deproteinized tissue extracts at pH 10, and the fluorescence measured in 3 N HCl (Bogdanski, Pletscher, Brodie & Udenfriend, 1956). Pooled 'brain stems' obtained from five rats were used for each 5-hydroxytryptamine estimation. Checks on recoveries of 50–100 ng of added amines were carried out in each experiment; the percentage recoveries were (means ± s.e. mean, number of experiments in brackets) dopamine, 75 ± 4 (12), noradrenaline, 61 ± 4 (10) and 5-hydroxytryptamine, 73 ± 2 (14).

Results

Effect of reserpine

The concentration of *p*-tyramine in the rat caudate nucleus was approximately 0.07% that of dopamine; *m*-tyramine in the caudate nucleus and β-phenylethylamine in the hypothalamus were present in even

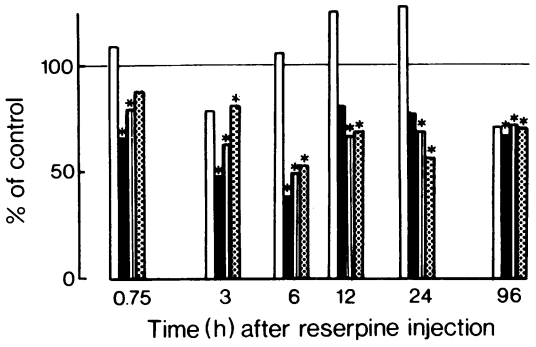

Figure 1 The effect of reserpine on the concentration of β-phenylethylamine (open columns) in the rat hypothalamus, and *p*-tyramine (solid columns), *m*-tyramine (vertically lined columns) and dopamine (dotted columns) in the rat caudate nucleus. Amine concentrations, expressed as percentages of control values, are plotted against the time (in hours) elapsed between drug administration and the death of the animal. The values are calculated from the results given in Table 1; *: significantly different from control.

smaller amounts (Table 1). Both *p*- and *m*-tyramine were depleted by the subcutaneous administration of reserpine; high doses (1 and 10 mg/kg) reduced the concentrations of these amines to less than 20% of those observed in the solvent-injected controls (Table 1). Similar reductions were observed for dopamine in the caudate nucleus, for noradrenaline in the thalamus, and for 5-hydroxytryptamine in the brain stem. However, the hypothalamic content of β-phenylethylamine was unaffected by these doses of reserpine (Table 1). A lower dose of reserpine had a less pronounced and more selective effect on the brain amine values. One day after the administration of 0.4 mg/kg of reserpine, *m*-tyramine and dopamine in the caudate nucleus, noradrenaline in the thalamus, and 5-hydroxytryptamine in the brain stem were still reduced significantly, but *p*-tyramine in the caudate nucleus was no longer significantly lower than the control value (Table 1). Neither *p*-tyramine nor *m*-tyramine was affected by reserpine at a dose of 0.2 mg/kg, but moderate reductions were observed in dopamine, noradrenaline and 5-hydroxytryptamine values.

The time course of the effect of a single dose of reserpine (0.4 mg/kg) is depicted in Table 1 and Figure 1. A significant decrease in the content of both *p*- and *m*-tyramine was apparent in the caudate nucleus as early as 45 min after administration of the drug, and continued for at least 6 h; after this period *p*-tyramine tended to return to its control value. The concentration of *m*-tyramine, however, remained significantly below the control value for at least 19 days. Dopamine in the caudate nucleus, noradrenaline in the thalamus,

Table 1 The effect of reserpine on the concentration of β-phenylethylamine (βPE), *p*-tyramine (PTA), *m*-tyramine (MTA), dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in different regions of the rat brain

Dose (mg/kg)	Time (h)	βPE Hypothalamus (ng/g)	PTA Caudate nucleus (ng/g)	MTA Caudate nucleus (ng/g)	DA Caudate nucleus (ng/g)	NA Thalamus (ng/g)	5-HT 'Brain stem' (ng/g)
Controls	–	2.52 ± 0.25 (16)	8.02 ± 0.76 (18)	2.25 ± 0.08 (15)	11,069 ± 689 (24)	615 ± 26 (15)	463 ± 23 (17)
0.2	24	–	7.35 ± 0.58 (3)	2.08 ± 0.12 (3)	8,245 ± 531 (5)‡	464 ± 44 (3)†	355 ± 44 (3)*
0.4	0.75	2.75 ± 0.23 (6)	5.31 ± 0.32 (6)‡	1.79 ± 0.16 (6)*	9,724 ± 1031 (6)	531 ± 16 (6)*	408 ± 16 (6)
0.4	3	1.99 ± 0.15 (3)	3.81 ± 0.23 (3)§	1.57 ± 0.08 (3)§	9,048 ± 503 (7)*	364 ± 23 (7)§	336 ± 15 (7)§
0.4	6	2.69 ± 0.69 (4)	3.09 ± 0.29 (4)§	1.11 ± 0.05 (4)§	5,895 ± 376 (4)§	377 ± 43 (4)§	374 ± 18 (4)†
0.4	12	3.17 ± 0.73 (3)	6.53 ± 0.27 (4)	1.51 ± 0.06 (4)§	7,611 ± 197 (4)§	274 ± 15 (4)§	278 ± 18 (4)§
0.4	24	3.22 ± 0.67 (8)	6.25 ± 0.78 (8)	1.56 ± 0.07 (8)§	6,372 ± 280 (10)§	264 ± 18 (6)§	282 ± 18 (8)§
0.4	96	1.81 ± 0.28 (4)	5.42 ± 0.52 (4)*	1.62 ± 0.07 (4)§	7,788 ± 208 (4)§	336 ± 16 (4)§	274 ± 12 (3)§
0.4	456	–	10.50 ± 0.32 (6)	1.95 ± 0.08 (6)*	11,029 ± 461 (6)	462 ± 13 (6)§	–
1	24	2.78 ± 0.66 (4)	1.41 ± 0.29 (7)§	0.39 ± 0.10 (8)§	1,260 ± 65 (12)§	85 ± 13 (11)§	115 ± 8 (10)§
10	24	5.1;2.6 (2)	0.64 ± 0.23 (5)§	0.21 ± 0.07 (5)§	469 ± 33 (10)§	39 ± 20 (5)§	40 ± 3 (10)§

Reserpine was administered subcutaneously. Results are given in ng/g of fresh tissue (± s.e mean, number of determinations in parentheses) and corrected for recoveries.
Student's *t*-test: *$P<0.05$, †$P<0.01$; ‡$P<0.005$, §$P<0.001$.

and 5-hydroxytryptamine in the brain stem, remained low for at least 4 days (Table 1, Figure 1). β-Phenylethylamine in the hypothalamus was unaffected by reserpine at any of the doses examined.

Effect of 6-hydroxydopamine

Rats that received a single intraventricular dose of 6-hydroxydopamine (250 μg) appeared sedated and lethargic when compared with vehicle-injected controls and showed piloerection. The effect was apparent one day after treatment and continued until the animals were killed on day 10. Spinal convulsions normally produced by decapitation were suppressed by the drug. The concentrations of *p*-tyramine, *m*-tyramine and dopamine in the caudate nucleus, and of noradrenaline in the hypothalamus were reduced significantly below control values by 6-hydroxydopamine; 5-hydroxytryptamine in the brain stem was unaffected (Table 2).

Discussion

Subcutaneous administration of reserpine elicited reduction in the concentrations of *p*-tyramine, *m*-tyramine, dopamine, noradrenaline and 5-hydroxytryptamine in the rat brain. Carlsson *et al.* (1963) observed that noradrenaline, dopamine and 5-hydroxytryptamine are taken up *in vitro* by bovine adrenal medullary granules by the same Mg^{2+}-ATP-dependent mechanism. The uptake is inhibited by reserpine, but the inhibitory effects of the drug may be counteracted by increasing the concentration of catecholamines in the medium. However, tyramine is taken up by a different mechanism which is only slightly activated by Mg^{2+}-ATP and is not sensitive to reserpine.

Our present results indicate that in the brain, the *p*- and *m*-isomers of tyramine may be stored by an intraneuronal reserpine-sensitive storage mechanism. This interpretation does not conform with the findings of Carlsson *et al.* (1963). However, their experiments were done *in vitro* on bovine adrenal medullary granules, while the present results were obtained *in vivo* in rat brain. Thus the earlier findings may not necessarily apply to the storage mechanisms for tyramine operating in the rat brain. Furthermore, the low concentrations of *p*-tyramine and *m*-tyramine which have been observed in the rat adrenal gland (10 ng/g and 5 ng/g respectively; Philips & Juorio, unpublished observations), suggest that the adrenals are unlikely to possess an extensive transport system for the tyramines. It could also be that the tyramines replace some of the catecholamines from their storage granules, and are then released by the nervous impulse to act as 'false transmitters' (Dorris & Shore, 1971).

The present results may also reflect the fact that *p*- and *m*-tyramine may be metabolically related to another amine which is stored in reserpine-sensitive granules. Analyses of rat urine have shown that both amines are related metabolically to dopamine (Boulton & Dyck, 1974). However, these studies are indicative of the metabolism of the body as a whole; they may not necessarily reflect the metabolism in the brain. Labelled *m*-tyramine was isolated from rat urine after an intraperitoneal injection of labelled dopamine and DOPA, but only very small amounts of the *para* isomer were found after the injection of dopamine, tyrosine and DOPA as substrates (Boulton & Dyck, 1974). On the other hand, *p*-tyramine does appear to be formed in larger amounts by hydroxylation of β-phenylethylamine than does *m*-tyramine (Boulton, Dyck & Durden, 1974), and may be synthesized primarily by this route. Thus for *p*-tyramine, at least, it is unlikely that the decrease in amine concentration elicited by reserpine is due to reduced availability of the metabolic precursor, since β-phenylethylamine is unaffected, at least in the hypothalamus, by reserpine. It seems more likely that *p*-tyramine is stored in a reserpine-sensitive granule. *m*-Tyramine may well be stored in a similar, or even the same compartment, but since it can be synthesized from DOPA and dopamine, and since its resynthesis rate after reserpine is similar to that of dopamine, it seems likely that this amine is associated with the dopamine compartment.

Table 2 The effect of 6-hydroxydopamine on the concentrations of *p*-tyramine (PTA), *m*-tyramine (MTA), dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in different regions of the rat brain

Treatment	*PTA Caudate nucleus (ng/g)*	*MTA Caudate nucleus (ng/g)*	*DA Caudate nucleus (ng/g)*	*NA Hypothalamus (ng/g)*	*5-HT 'Brain stem' (ng/g)*
Controls	10.55 ± 1.89 (5)	2.07 ± 0.16 (5)	8439 ± 241 (5)	1356 ± 171 (3)	441,455 (2)
6-Hydroxydopamine	3.57 ± 0.56 (7)*	0.87 ± 0.12 (7)‡	3149 ± 219 (7)‡	480 ± 38 (7)†	408 ± 21 (6)

Animals treated with 6-hydroxydopamine (250 μg of base) were killed 10 days after a single intraventricular injection of the drug. Results are given in ng/g of fresh tissue (± s.e. mean, number of determinations in parentheses) and corrected for recoveries.
Student's *t*-test: *$P < 0.01$; †$P < 0.005$; ‡$P < 0.001$.

It is somewhat surprising that the octopamines, which are produced metabolically by β-hydroxylation of the tyramines (Snyder, Glowinski & Axelrod, 1965; Anagnoste & Goldstein, 1967), are not similarly depleted by reserpine, as Harmar & Horn (1976) have reported that in mouse whole brain, octopamine is not reduced significantly 18 h after an intraperitoneal injection of reserpine (5 mg/kg).

The effect of 6-hydroxydopamine on the concentrations of *p*- and *m*-tyramine may also be explained as either a direct effect, in which specific tyraminergic neurones may be destroyed, or as an indirect effect, in which metabolic precursors of the tyramines are depleted by destruction of dopaminergic neurones. From the present experiments it is not possible to differentiate between these various possibilities.

It is interesting to note that the cerebral regional concentrations of dopamine and *p*- and *m*-tyramine do not change in parallel in all instances. Danielson, Wishart & Boulton (1976) have recently shown that 30 min after an injection of (+)-amphetamine sulphate (5 mg/kg), the concentration of *p*-tyramine in the striatum was reduced to about half the control value, while that of *m*-tyramine increased approximately two-fold. Under similar experimental conditions dopamine concentrations increased only slightly (Aylmer, Steinberg & Webster, 1975). These observations may reflect the fact that amphetamine is able to compete with β-phenylethylamine for the hydroxylating enzyme, thus reducing the synthesis of *p*-tyramine from β-phenylethylamine, while *m*-tyramine synthesis is enhanced somewhat by the slightly increased concentrations of dopamine.

β-Phenylethylamine is unaffected by reserpine. Because it is lipid-soluble, it readily crosses the blood-brain barrier (Oldendorf, 1971), and is found in all subcellular fractions of the rat brain, including the synaptosomes (Boulton & Baker, 1975). β-Phenylethylamine may be stored in reserpine-resistant granules. Alternatively it may not be stored in granules at all, but may exist throughout the cell and be more or less freely exchangeable with extracerebral β-phenylethylamine. Because of its non-polar nature, and hence its ability to permeate cell membranes, it seems that the latter is more likely. It has been proposed (Boulton, 1976) that β-phenylethylamine may function as an activator of the synaptic area. As such, it may function to maintain the synapse in a state of activation insufficient to create postsynaptic effects but necessary for propagation of nervous impulses by more conventional neurotransmitters. Such a role would obviate the need for β-phenylethylamine to be stored in granules or to be otherwise associated with membranous structures.

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