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THE EFFECT OF γ-HYDROXYBUTYRATE ON MOUSE STRIATAL TYRAMINE, DOPAMINE AND HOMOVANILLIC ACID

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1 The concentrations of *p*- and *m*-tyramine, dopamine and homovanillic acid were measured in the mouse striatum following the subcutaneous injection of γ-hydroxybutyrate; their control levels were 19.8, 6.3, 9600 and 1130 ng/g respectively.

2 The administration of 500–1000 mg/kg of γ-hydroxybutyrate produced a reduction in *p*-tyramine that lasted at least 8 h. *m*-Tyramine and dopamine were significantly increased for at least 4 h. The levels of homovanillic acid were increased at 1 and 2 h after drug administration.

3 These experiments strongly suggest that the increases in dopamine turnover produced by γ-hydroxybutyrate caused reciprocal changes in striatal tyramine that are similar to those produced by drugs or treatment that increase dopamine turnover and tyrosine hydroxylase activity.

Introduction

Earlier work from this laboratory has shown that the increases in dopamine turnover produced by blockade of dopamine receptors, (+)-amphetamine-induced dopamine release and re-uptake inhibition, or during the first day after electrolytic lesioning of the pars compacta of the substantia nigra, occur concomitantly with a reduction in the concentration of striatal *p*-tyramine and no change or increase in *m*-tyramine (Juorio, 1977a,b; Juorio & Jones, 1981). It has been known for some time that sodium γ-hydroxybutyrate or its precursor γ-butyrolactone cause a dose-dependent increase in the cerebral concentration of dopamine and its acid metabolites (Gessa, Vargiu, Crabai, Boero, Caboni & Camba, 1966; Hutchins, Rayevsky & Sharman, 1972) and a reduction in the firing rate of dopaminergic neurones (Roth, Walters & Aghajanian, 1973). The present study examines the effect of γ-hydroxybutyrate on the interrelation between brain dopamine turnover and the concentration of the *p*- or *m*-isomers of tyramine. Changes in homovanillic acid concentrations have been taken as an indicator of dopamine turnover.

Methods

Sodium γ-hydroxybutyrate (Sigma) was dissolved in 0.9% w/v NaCl solution (saline) and injected subcutaneously. Male albino Swiss mice (18–22 g body wt.) were killed by decapitation, the brain was removed rapidly and the striatum, consisting mainly of the head of the caudate nucleus and including some of the underlying putamen (approximate weights were between 27 to 35 mg), was dissected out. Striata from three mice were pooled, immediately frozen on dry ice, weighed and homogenized in 0.1 N HCl containing disodium edetate (EDTA 1 mg/ml). The amines in the tissue homogenate were derivatized with 5-dimethylamino-1-naphthalenesulphonyl (dansyl) chloride and the resultant derivatives extracted into toluene : ethyl acetate (9 : 1), evaporated to a small volume, separated chromatographically in two different monodimensional systems (system 1, toluene : ethyl acetate, 5 : 2; system 2, toluene : triethylamine : methanol, 50 : 5 : 1). The estimations of *p*- and *m*-tyramine were carried out by a high resolution mass spectrometric integrated ion current technique using deuterated *p*- or *m*-tyramine as internal standards. Complete details concerning this procedure have been described (Philips, Durden & Boulton, 1974; Philips, Davis, Durden & Boulton, 1975). Dopamine was estimated by the fluorimetric method proposed by Laverty & Sharman (1965), using the pooled striata of two mice. Dopamine was separated on a Dowex 50W × 4 ion exchange chromatography column, then acetylated, its fluorophore developed by condensation with 1,2-diaminoethanol, and estimated by spectrophotofluorimetry. Checks on the recoveries of 100 ng of added dopamine were carried out in each experiment; the percentage recovery was 85% ± 3 (7) (mean ± s.e.mean; number of experiments in parentheses). The results were corrected for losses. Homovanillic acid was estimated in the pooled striata of five mice. The tissues were homogenized in 0.1 N HCl, deproteinized with 0.4 N perchloric acid, extracted with butyl acetate and from it into 0.05 M Tris

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buffer, and estimated fluorimetrically (Andén, Roos & Werdinius, 1963). Checks on recoveries of 200 ng of homovanillic acid were carried out in every experiment; the percentage recovery was 74 ± 2 (11) (mean ± s.e.mean; number of experiments in parentheses) and the results corrected.

Results

The subcutaneous administration of 500 mg/kg of γ-hydroxybutyrate produced a significant increase in striatal *m*-tyramine after 1 h and a smaller, but statistically significant, increase (152% of controls) in homovanillic acid (Table 1). No changes were observed in *p*-tyramine at 1 h, but 2 h after the striatal levels of *p*-tyramine were reduced to about 60% of controls and a statistically non-significant increase (122% of controls) was observed for *m*-tyramine (Table 1). The administration of a larger dose of γ-hydroxybutyrate (1000 mg/kg) also produced a reduction in mouse striatal *p*-tyramine that was observed 2 h after drug administration (Table 1). At 8 h the *p*-tyramine concentration had started to recover and was 81% of its control value. The concentration of *m*-tyramine was markedly increased by this treatment (to about 270% of control values) after 1 or 2 h and then recovered to 121% of controls after 8 h (Table 1).

Mouse striatal levels of dopamine were significantly increased (165–141% of controls) at 1 or 2 h after the administration of γ-hydroxybutyrate (1000 mg/kg) (Table 1). At this time the increases in striatal homovanillic concentration were maximal (159–297% of controls). By 8 h after drug administration homovanillic acid had returned to near its control value (Table 1).

Discussion

The parenteral administration of γ-hydroxybutyrate produces a reduction in animal locomotor activity and depression of the central nervous system that can reach general anaesthesia (Rubin & Giarman, 1947; Laborit, Jouany, Gerard & Fabiani, 1960). The relatively high doses (500–1000 mg/kg) required to produce anaesthesia caused not only an increase in brain dopamine (Gessa *et al.*, 1966), but also an activation of tyrosine hydroxylase activity (Morgenroth, Walters & Roth, 1976) and increases in 3,4-dihydroxyphenylacetic and homovanillic acids (Hutchins *et al.*, 1972), and reductions in the firing rate of presumed dopaminergic nigral neurones (Walters, Roth & Aghajanian, 1973). Such results indicate that γ-hydroxybutyrate produces changes in dopamine metabolism that are similar to those observed within one day after lesioning the pars compacta of the substantia nigra (Walters, Roth & Aghajanian, 1973; Faull & Laverty, 1969). The experiments described here confirm these increases in dopamine turnover and further show that these persist for at least 4 h and return to near control values by 8 h following drug administration. In addition, the increases in dopamine turnover are accompanied by a reduction in striatal *p*-tyramine and an increase in *m*-tyramine that becomes apparent within 2 h after drug administration. This differential effect on the tyramines persists for 4 to 8 h at which time the homovanillic acid concentration has returned to normal or somewhat below normal levels.

The activation of tyrosine hydroxylase activity that occurs concomitantly with increases in dopamine turnover favours the hydroxylation of *p*-tyrosine into DOPA that in turn is decarboxylated to form dopamine (Bartholini & Pletscher, 1969). This chain of events would reduce the availability of *p*-tyrosine

Table 1 Effects of the subcutaneous administration of γ-hydroxybutyrate on mouse striatal *p*-tyramine (*p*-TA), *m*-tyramine (*m*-TA), dopamine (DA) and homovanillic acid (HVA)

Dose (mg/g)	*Time* (h)	*p-TA* (ng/g)	*m-TA* (ng/g)	*DA* (ng/g)	*HVA* (ng/g)
—	—	20.4 ± 0.8 (9)	6.8 ± 0.9 (9)	10,050 ± 500 (9)	1060 ± 80 (16)
500	1	20.0 ± 0.5 (5)	12.0 ± 1.4 (5)**	—	1610 ± 170 (7)**
500	2	12.2 ± 1.0 (7)***	8.3 ± 0.4 (7)	—	1610 ± 20 (4)***
1000	1	17.8 ± 3.4 (5)	18.6 ± 2.4 (5)***	16,620 ± 700 (5)***	1690 ± 210 (4)*
1000	2	10.6 ± 1.9 (6)***	18.1 ± 2.2 (6)***	14,200 ± 1160 (5)**	3150 ± 450 (6)***
1000	4	9.0 ± 0.6 (5)***	10.0 ± 0.8 (5)*	11,590 ± 120 (4)	1640 ± 120 (5)***
1000	8	16.5 ± 0.5 (5)**	7.6 ± 0.4 (5)	9,430 ± 440 (8)	890 ± 60 (14)

Values are means (± s.e.mean; number of experiments in parentheses) in ng/g of fresh tissue.
Student's *t* test: *$P < 0.025$; **$P < 0.01$; ***$P < 0.001$.

and in addition, with the relatively low affinity (high *Km*) that this amino acid possesses toward the enzyme 1-aromatic amino acid decarboxylase (Nagatsu, Levitt & Udenfriend, 1964), it would lead to a reduction in the concentration of *p*-tyramine. The inhibition of tyrosine hydroxylase elicited directly by α-methyl-*p*-tyrosine (Spector, Sjoersma & Udenfriend, 1965) or indirectly by a dopamine receptor agonist (Roos, 1969) produced the opposite effect, that is reduction in the formation of DOPA and dopamine and increases in *p*-tyrosine and *p*-tyramine (Juorio, 1979). Also, the accumulation of rat brain dopamine following treatment with γ-hydroxybutyric acid was inhibited by the administration of dopamine receptor agonists (Handforth & Sourkes, 1975).

Nembutal which also possesses general anaesthetic action has no significant effect on the levels of brain *p*- or *m*-tyramine (A.V. Juorio, unpublished).

A mechanism that could underlie these interactions would be the synthesis of *p*-tyramine and *m*-tyramine by alternative pathways and that one or the other act postsynaptically to alter the action of the transmitter. Neurophysiological experiments have shown that *p*- and *m*-tyramine isomers potentiate the reduction in the neuronal firing rate produced by the iontophoretic application of dopamine (Jones & Boulton, 1980). This may be due to a postsynaptic effect.

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