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Tyrosine hydroxylase activity in rat brain regions after chronic treatment with ±-propranolol

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Rats were injected twice daily with ±-propranolol (6 mg kg^{-1} day^{-1}) for 14 days and killed 16 h after the final injection. Tyrosine hydroxylase activity was measured in both soluble and particle-bound forms in various brain regions. The activity of the soluble enzyme was not significantly altered by propranolol treatment in any of the brain regions studied. The tyrosine hydroxylase activity in the particulate fraction was significantly increased in corpus striatum and unchanged in other brain regions. The propranolol concentrations in the various brain regions in this chronic study were far lower than necessary to produce a significant change in tyrosine hydroxylase activity in acute experiments. It was concluded that chronic propranolol treatment produces a persistent increase in bound tyrosine hydroxylase activity in rat corpus striatum.

The current literature on the effect of propranolol on brain catecholamine concentrations is confusing. There is evidence that it affects brain catecholamine concentrations. Brunner, Hedwall & others (1966) reported a decrease in rat brain noradrenaline after chronic propranolol treatment (10 mg kg^{-1} daily for 4 days), although Laverty & Taylor (1968) were unable to find significant changes in rat brain noradrenaline, dopamine, normetanephrine, 3-methoxytyramine or homovanillic acid after either subcutaneous (10 mg kg^{-1} daily for 4 days) or oral administration. Mazurkiewicz-Kwilecki & Romagnoli (1970) found no change in rat brain catecholamine content after 9 weeks treatment with 6 or 12 mg kg^{-1} day^{-1}. In contrast, Aro & Blinge (1971) found an increase in brain noradrenaline concentrations of 15–30% without change in dopamine after 7 days treatment with 10 or 15 mg kg^{-1} day^{-1} of any of four β-adrenoceptor blocking agents including propranolol. A single intraperitoneal injection of propranolol (10 or 30 mg kg^{-1}) produced no significant change in the concentrations of noradrenaline, dopamine or three catecholamine metabolites (Laverty & Taylor, 1968) in either whole rat brain or in various brain regions. In the cat, intraventricular infusion of propranolol led to a decrease in hindbrain noradrenaline and an increase in telencephalic noradrenaline (Kelliher & Buckley, 1970). A single injection of propranolol (10 mg kg^{-1}) did not alter dopamine or noradrenaline turnover in whole rat brain (Andén & Strombom, 1974). Similarly, there was no significant change in the rate of synthesis of catecholamines from L-[^{3}H]tyrosine in whole rat brain after daily administration of 6 mg kg^{-1} propranolol for up to 6 weeks (Mazurkiewicz-Kwilecki, Filczewski & Peters, unpublished).

Sullivan, Segal & others, (1972) studied the effect of acute propranolol treatment on the activity of tyrosine hydroxylase, the enzyme involved in the rate controlling step in catecholamine biosynthesis (Levitt, Spector & others, 1965). A single intraperitoneal injection of propranolol (15–45 mg kg^{-1}) produced a short lasting increase in tyrosine hydroxylase activity in rat corpus striatum. The enzyme activity was significantly greater than in control animals 1 and 2 h after a 30 mg kg^{-1} injection with a return to control values at 4 h. In contrast, midbrain tyrosine hydroxylase showed no change in total measurable activity following propranolol administration. The increased striatal tyrosine hydroxylase activity was paralleled by a behavioural depression.

We have investigated the action of propranolol on several aspects of catecholamine synthesis and utilization. The present study reports the effect of repeated administration of small doses of propranolol on the activity of tyrosine hydroxylase in various regions of rat brain.

MATERIALS AND METHODS

Male Sprague-Dawley rats (150–175g), randomly divided into two groups, received intraperitoneal injections of either ±-propranolol (3 mg kg^{-1} in 0·9% saline) or the saline vehicle alone at 9 00 a.m. and 4 00 p.m. daily for 14 days and the animals killed 16 h after the final injection. Two similar groups of rats were given a single intraperitoneal injection of either propranolol (3 mg kg^{-1}) or saline and killed 1 h later. The brains were removed, rinsed briefly with ice cold saline, blotted dry and placed on a glass plate cooled in crushed ice. The corpus striatum, cerebral cortex, midbrain-hypothalamus, pons-medulla and cerebellum were dissected according to Glowinski & Iversen (1966). Brain regions were homogenized in 2 ml ice cold 0·28 M sucrose using a Polytron PT-10 homogenizer (Brinkmann Instruments) at slow speed for 30 s. The homogenate was centrifuged at 0–4° for 10 min at 1000*g* to sediment the nuclear fraction and then at 20 000*g* for 1 h to obtain the P2 fraction (Gray & Whittaker, 1962) and a supernatant. The P2 pellet was taken up in 2 ml water and tyrosine hydroxylase activity assayed in both the P2 fraction (described as the particulate enzyme) and in the final supernatant (described as the soluble enzyme) by the method of McGeer, Gibson & McGeer (1967) with slight modifications.

Incubation mixtures consisted of 2 nmol L-tyrosine containing 250 000 $dmin^{-1}$ L-[U-^{14}C]tyrosine (507 mCi mM^{-1}; Amersham-Searle); 500 nmol NSD 1034 (Smith and Nephew Research); 500 nmol 6,7-dimethyl-5,6,7,8-tetrahydropterin ($DMPH_4$; Calbiochem); 60 μmol 2-mercaptoethanol; 250 nmol $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$; 100 μmol sodium acetate buffer, pH 6·0 and 0·1 ml of enzyme source in a total volume of 0·5 ml. After incubation for 30 min at 37° the L-[^{14}C]dopa produced was isolated on an alumina column. Radioactivity measurements were made in a Nuclear Chicago Mark I liquid scintillation spectrometer.

For the assay of propranolol, brain regions were homogenized in 2 ml 0·4 N perchloric acid, centrifuged at 1000*g* for 10 min and an aliquot of the supernatant used to isolate propranolol according to Stock & Westermann (1965) for the assay of KÖ 592 in brain tissue. Propranolol was measured fluorometrically in the final extract at 293 nm activation and 359 nm emission wavelengths (uncorrected) in an Aminco-Bowman spectrophotofluorometer.

RESULTS

Table 1 shows the propranolol concentration in various regions of rat brain after chronic and acute intraperitoneal administration of the drug. One hour after a single injection of 3 mg kg^{-1}, the concentrations were highest in the striatum and cortex and lowest in the midbrain-hypothalamus. A second group of animals killed 16 h after a single 3 mg kg^{-1} injection did not show detectable concentrations of the drug in any of the brain regions. These results are in general agreement with those of Laverty & Taylor (1968). The brain extract of the group of rats given twice daily injections of propranolol for 14 days showed a slightly higher fluorescence at the usual wavelengths

Table 1. *Propranolol concentrations in various regions of rat brain after acute and chronic administration of ±-propranolol.* Values are given as mean ± s.e.m. for groups of at least 6 rats. The animals were killed 1 or 16 h after the final propranolol injection.

Treatment	Time (h)	Propranolol (ng g^{-1} wet weight) Cerebellum	Cortex	Corpus striatum	Midbrain-hypothalamus	Pons-medulla
3 mg kg^{-1}	1	1540 ± 110	2210 ± 40	1820 ± 30	1110 ± 30	1360 ± 40
3 mg kg^{-1}	16	< 20	< 20	< 20	< 20	< 20
14 days, 2 × 3 mg kg^{-1}	16	50 ± 20	75 ± 25	86 ± 33	41 ± 15	35 ± 20

of the propranolol peak than did an identical extract prepared from animals receiving the vehicle, but in none was the fluorescence reading sufficiently above blank for a definite identification of the propranolol spectra to be made. The propranolol concentrations, calculated by assuming the difference in fluorescence between control and experimental animals to be due solely to propranolol, showed approximately the same distribution as in the acute experiment. Although there is some evidence of a slight accumulation of propranolol the brain concentrations after chronic treatment are far less than those shown to produce the acute effects on tyrosine hydroxylase activity reported by Sullivan & others (1972).

Table 2. *Effect of chronic propranolol treatment on tyrosine hydroxylase activity in various regions of rat brain.* Values are given as mean ± s.e.m. for groups of 6–8 rats. The percentage of control values is given in brackets. The experimental animals received twice daily injections of propranolol (6 mg kg^{-1} day^{-1}) for 14 days. Control animals received the saline injections on the same schedule. All animals were killed 16 h after the final injection.

Enzyme source	Treatment	Tyrosine hydroxylase (nM g^{-1} h^{-1}) Cortex	Striatum	Midbrain-hypothalamus	Pons-medulla
P2 Fraction	Saline	6·08 ± 0·52	214 ± 18	31·0 ± 3·0	21·6 ± 0·8
	Propranolol	6·84 ± 0·44	284 ± 22 ($P < 0·05$)	29·0 ± 1·4	23·2 ± 1·6
		(113)	(132)	(94)	(107
Supernatant	Saline	3·10 ± 0·26	43·6 ± 2·0	27·4 ± 2·6	11·8 ± 1·0
	Propranolol	3·00 ± 0·26	47·4 ± 4·8	26·0 ± 0·8	10·0 ± 1·4
		(97)	(109)	(95)	(85)

Table 2 shows the effect of chronic propranolol treatment on tyrosine hydroxylase activity in rat brain. When the particulate form of the enzyme was assayed in the presence of a pteridine co-factor the tyrosine hydroxylase activity was significantly elevated in striatum, slightly but not significantly increased in cortex and unchanged in midbrain-hypothalamus and pons-medulla. Soluble tyrosine hydroxylase activity was not significantly changed by chronic propranolol treatment.

One hour after a single intraperitoneal injection of propranolol (15 mg kg^{-1}) there was a significant decrease in tyrosine hydroxylase activity in the P2 fraction prepared from pons-medulla (Table 3). The enzyme activity was slightly but not significantly increased in striatum and unchanged in cerebral cortex and midbrain-hypothalamus. A single 3 mg kg^{-1} injection of propranolol had no effect on the tyrosine hydroxylase activity in any of the brain regions studied. Body and brain weights were not affected by either acute or chronic drug treatment.

Table 3. *Effect of a single propranolol injection on tyrosine hydroxylase activity in various regions of rat brain.* Groups of 6 or more rats received either 15 mg kg^{-1} propranolol or saline and were killed 1 h later. Results are given as mean ± s.e.m. Tyrosine hydroxylase was assayed in the P2 fraction in the presence of $DMPH_4$.

Brain region	Tyrosine hydroxylase (nM g^{-1} h^{-1}) Control	Experimental	% Control
Cortex	6·4 ± 0·4	6·7 ± 0·5	105
Striatum	216 ± 10	242 ± 14	112
Midbrain-hypothalamus	27·4 ± 1·8	28·0 ± 1·0	102
Pons-Medulla	20·6 ± 0·7	18·2 ± 0·4 ($P < 0·05$)	88

DISCUSSION

Sullivan & others (1972) have shown that a single injection of propranolol led to a significant increase in striatal tyrosine hydroxylase activity (16 ± 3%, at 15 mg kg^{-1}; 28 ± 2%, at 30 mg kg^{-1}) after 1 h followed by a return to control concentrations at 4 h. The increase could not be blocked by cycloheximide. Midbrain tyrosine hydroxylase activity was not affected by propranolol treatment. Both the rapid increase in enzyme activity and lack of blockade of the increase by an inhibitor of protein synthesis suggest that enzyme induction was not involved. It was concluded that a change in the physical state of the enzyme at the nerve terminals was responsible for the increased enzyme activity rather than an increased enzyme protein. Our results show that 1 h after a 15 mg kg^{-1} injection of propranolol tyrosine hydroxylase activity in the P2 fraction from striatum was slightly but not significantly elevated (+12%), ($P < 0·1 > 0·05$). The tyrosine hydroxylase activity was unchanged in midbrain and showed a small but significant decrease (−12%) in pons-medulla ($P < 0·05$).

After chronic propranolol treatment (6 mg kg^{-1} day^{-1} for 14 days) the tyrosine hydroxylase activity in rat corpus striatum was significantly increased in the P2 fraction when measured 16 h after the last injection. At this time the drug could not be clearly identified in any of the brain regions studied, although fluorescence measurements suggested that a small amount was present. After a single injection, the

enzyme activity was increased only during the period at which brain propranolol levels were at their highest. Concentrations 1 h after a single 3 mg kg^{-1} injection were at least 20 times greater than at the time of death in the chronic experiment. However, a single 3 mg kg^{-1} injection of propranolol had no measurable effect on brain tyrosine hydroxylase activity. Only when the dose was increased to 15 mg kg^{-1} was there any evidence of an enhanced striatal tyrosine hydroxylase activity in acute experiments. The persistent increase in striatal tyrosine hydroxylase activity found after chronic treatment was therefore unlikely to be due to the presence of propranolol in the tissue. The increased enzyme activity after chronic treatment may be the result of an increase in enzyme protein rather than a transient change in the physical state of existing enzyme. This possibility is supported by evidence that tyrosine hydroxylase is an inducible enzyme in several tissues (Thoenen, 1974) including brain (Mueller, Thoenen & Axelrod, 1969; Thoenen, 1970; Mandell, 1970; Lamprecht, Eichelman & others, 1972).

Propranolol may be effective in the treatment of Parkinson's disease (Owen & Marsden, 1965; Marsden & Owen, 1966). It is therefore interesting that the only region of brain in which chronic propranolol treatment causes a significant change in tyrosine hydroxylase activity was the corpus striatum since this region contains an unusually high concentration of dopamine (Bertler & Rosengren, 1959).

Propranolol is reported to have therapeutic potential for the treatment of psychotic and anxiety states (Wheatley, 1969; Atsmon, Blum & others, 1971; Carlsson & Johansson, 1971; Atsmon, Blum & others, 1972). Its ability to influence brain tyrosine hydroxylase activity may be relevant to its clinical mechanism of action. Moreover, as observed by Sullivan & others (1972), propranolol, like reserpine, is reported to produce severe depression of mood in some patients (Waal, 1967) and reserpine has been shown to produce a similar increase in brain tyrosine hydroxylase activity (Segal, Sullivan & others, 1971; Mueller & others, 1969). It is conceivable that the drug-induced alterations in tyrosine hydroxylase activity are involved in the mood changes experienced by some users of these drugs.

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