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Activation of tyrosine hydroxylase in the frontal cortex by phentolamine and prazosin†

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Tyrosine hydroxylase is considered to be the rate-limiting enzyme in the pathway concerned with synthesis of catecholamines. Short term activation of tyrosine hydroxylase can occur following nerve stimulation or stress. This has been demonstrated in a number of systems, including the guinea-pig vas deferens preparation after stimulation of the hypogastric nerve (Weiner et al 1978), the rat hippocampus on stimulation of the locus coeruleus (Roth et al 1975a), the rat striatum following electrical stimulation of the nigro-neostriatal pathway (Roth et al 1975b), the rat adrenal gland and striatum after electroconvulsive shock (Masserano et al 1981) and the rat striatum following the administration of dopamine receptor blocking agents (Zivkovic et al 1974). In these studies the increased activity of tyrosine hydroxylase is associated with an increased affinity (reduced K_m) of the enzyme for its pterin cofactor.

The purpose of the present experiments was to determine if a similar activation of tyrosine hydroxylase from a predominantly noradrenergic brain region (frontal cortex) can occur following the administration of the specific α-adrenoceptor blocking agents, phentolamine and prazosin.

Methods

Male rats (Charles River Sprague-Dawley), 175–225 g, housed with free access to food and water were used. The rats received either phentolamine 5 mg kg^{-1} i.p. or prazosin 10 mg kg^{-1} i.p. in a volume of 0·5–1·0 ml distilled water. Control animals received a similar volume of distilled water without drug. Sixty minutes after the injection of either drug or vehicle the animals were decapitated and the brain areas were dissected. The tissues were weighed and homogenized in ten volumes (striatum), eight volumes (substantia nigra), five volumes (locus coeruleus), four volumes (frontal cortex), or three volumes (hippocampus) of 50 mM Tris-acetate buffer (pH 6·2) containing 0·2% Triton X-100. The homogenates were centrifuged at 40 000 *g* for 30 min at 4 °C and 10 μl of the supernatant was assayed for tyrosine hydroxylase activity. The tyrosine hydroxylase activity was determined by a modification of the method Kapatos & Zigmond (1979). The standard assay mixture contained: 120 mM TES buffer (pH 6·2), 5 mM ascorbic acid, 1300 units of catalase, 0·25 mM 2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine HCl (6-$MePtH_4$), and 0·1 mM L-[1-^{14}C]tyrosine (spec. act. approximately 50 mCi $mmol^{-1}$). Neither phentolamine nor prazosin, in concentrations ranging from 1 μM to 1 mM, had any effect on tyrosine hydroxylase activity when added to the enzyme assay. When kinetic studies were performed, the 6-$MePtH_4$ concentration was varied from 0·033 mM to 1 mM. The results were analysed according to the method of Lineweaver and Burk and the kinetic constants were determined by linear regression analysis.

Results and discussion

Initially, the activity of tyrosine hydroxylase from the frontal cortex and four additional brain regions was measured in the presence of subsaturating concentrations of 6-$MePtH_4$ (0·25 mM) 60 min after the intraperitoneal injection of phentolamine (5 mg kg^{-1}) (Table 1).

Table 1. The effect of phentolamine (5 mg kg^{-1} i.p.) on brain tyrosine hydroxylase activity assayed 60 min after administration. The results are presented as n mol h^{-1} mg^{-1} protein and are the means of five experiments ± s.e.m. All values were determined at 0·1 mM L-[1-^{14}C]tyrosine and 0·25 mM 6-$MePtH_4$.

Brain area	Tyrosine hydroxylase activity (nmol h^{-1} mg^{-1} protein) Control	Phentolamine
Frontal cortex	0·17 ± 0·01	0·25 ± 0·01*
Striatum	6·78 ± 0·35	6·66 ± 0·69
Locus coeruleus	0·68 ± 0·03	0·68 ± 0·06
Hippocampus	0·12 ± 0·01	0·16 ± 0·02*
Substantia nigra	4·01 ± 0·36	4·24 ± 0·26

* Significantly different from control, $P < 0·05$.

Only in the cortex and the hippocampus was the activity of tyrosine hydroxylase from phentolamine-treated animals found to be significantly greater than that from the control animals. In prazosin-treated animals (10 mg kg^{-1}), the activity of tyrosine hydroxylase from the cortex and striatum was measured and, as with phentolamine treatment, only cortical tyrosine hydroxylase activity increased following prazosin. Thus, both adrenoceptor blockers appear to produce an activation of tyrosine hydroxylase in the cortex. This observation is consistent with that of Gysling & Roth

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(1981) who reported an activation of tyrosine hydroxylase from the hippocampus, a predominantly noradrenergic region, following treatment with the α_2-adrenoceptor antagonist piperoxan.

To investigate further the nature of this apparent activation, the kinetic properties of cortical tyrosine hydroxylase from animals receiving either phentolamine, prazosin or distilled water were compared. Fig. 1 illustrates tyrosine hydroxylase activity in the frontal cortex measured in the presence of different amounts of the pterin cofactor, 6-MePtH$_4$. While only the data for prazosin are shown, the phentolamine results were nearly identical. This activation of cortical tyrosine hydroxylase by prazosin is accompanied by a significant decrease in the apparent K_m for 6-MePtH$_4$ from 0·30 mM to 0·20 mM. The activation of cortical tyrosine hydroxylase by phentolamine is accompanied by a shift in the apparent K_m for 6-MePtH$_4$ from 0·30 to 0·21 mM (data not shown). Neither drug treatment significantly

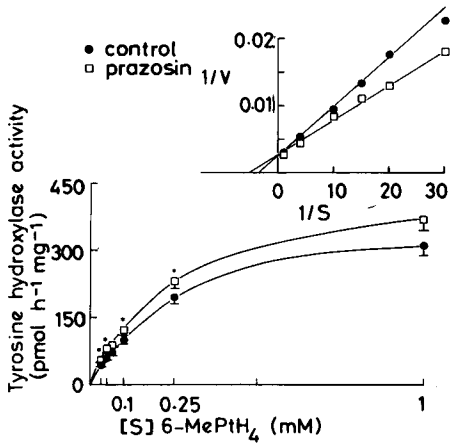

FIG. 1. Kinetic analysis of the effects of prazosin on tyrosine hydroxylase activity in the frontal cortex at various cofactor concentrations. The results are means from 6 experiments ± s.e.m. All values were determined at 0·1 mM L-[1-^{14}C]tyrosine. The K_m values were determined from the intercept of the double-reciprocal plot with the abscissa by means of linear regression analysis. In the same manner, the V_{max} values were determined from the intercept of the ordinate.

changed the apparent V_{max} with respect to 6-MePtH$_4$ from that of the control value. The apparent K_m values for tyrosine hydroxylase in the frontal cortex after phentolamine and prazosin treatment (0·21 and 0·20 mM, respectively) are within the range of previously reported K_m values (0·11 to 0·29 mM) resulting from such treatments as nerve stimulation or neuroleptic administration using tissue from striatum (Zivkovic et al 1974; Lovenberg & Bruckwick 1975), nucleus accumbens (Zivkovic et al 1975), hypothalamus and brain stem (Zikovic et al 1974), or vas deferens (Weiner et al 1978). Although the K_m value obtained for control cortical tissue (0·30 mM) was significantly different from that for cortex from treated animals, it was still considerably below the range of 0·47 to 0·80 mM reported for other brain regions and peripheral tissues from untreated animals. The relatively low K_m value for control cortical tissue does not appear to be due to the assay system used since tyrosine hydroxylase from control striatal tissue had a K_m value of 0·58 mM for 6-MePtH$_4$ when measured with this assay. These data indicate that tyrosine hydroxylase in the frontal cortex of 'control' animals is maintained in a higher state of activation than tyrosine hydroxylase in other tissues in the central or peripheral nervous systems.

The results presented are consistent with earlier studies using dopamine antagonists that activate tyrosine hydroxylase only in the striatum, a dopaminergic region. We found that the α-adrenoceptor blockers activated tyrosine hydroxylase only in the cortex and hippocampus, predominantly noradrenergic regions. Both phentolamine and prazosin produced an activation of tyrosine hydroxylase with a reduction in the apparent K_m for the pterin cofactor. A number of similarities exist between the acute activation of tyrosine hydroxylase in central dopamine and noradrenaline neurons, including an activation of tyrosine hydroxylase in the hippocampus, which contains noradrenaline-containing nerve terminals (Roth et al 1975a) after stimulation of the locus coeruleus, and an acute activation of tyrosine hydroxylase in the striatum, which is rich in dopamine-containing nerve terminals (Roth et al 1975b), after stimulation of the substantia nigra. In addition, administration of the α-adrenoceptor antagonist, phentolamine (Dairman et al 1968), produces an increase in turnover of noradrenaline in the brain, whereas an increase in turnover of dopamine in the striatum occurs after the administration of neuroleptic drugs such as haloperidol (Andén et al 1970a). In contrast, the α-adrenoceptor agonist clonidine has been shown to decrease central noradrenaline turnover (Andén et al 1970b), while the dopamine agonist apomorphine reduces central dopamine turnover (Andén et al 1967). The activation of tyrosine hydroxylase resulting from dopamine antagonist administration can be blocked by prior administration of the dopamine agonist, apomorphine (Zivkovic et al 1974). It remains to be determined whether noradrenaline agonists can block the activation of tyrosine hydroxylase in the frontal cortex produced by phentolamine or prazosin. Further investigation is required to determine the mechanism by which these antagonists activate tyrosine hydroxylase in central noradrenergic nerve terminals.

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