*Short communications*

# Catecholamine concentrations and the activity of tyrosine hydroxylase after an increase in the concentration of tyrosine in rat tissues

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The concentrations of tyrosine in rat plasma and brain were increased 2–7 fold by the administration of either L-tyrosine or cycloheximide. Under these conditions catecholamine concentrations in the brain and the heart remained unchanged even when the rats were maintained in a cold environment to increase catecholamine turnover. The data are interpreted to mean that an increase in the tyrosine concentration in the tissues does not result in an *in vivo* substrate inhibition of tyrosine hydroxylase.

The finding that tyrosine hydroxylase was the enzyme responsible for the rate limiting step in the production of catecholamines (Levitt, Spector, Sjoerdsma & Udenfriend, 1965) has focussed considerable attention on factors which serve to modulate the activity of this enzyme. The *in vivo* activity of tyrosine hydroxylase and the rate of catecholamine synthesis vary in direct relation to the degree of sympathetic nerve activity (Gordon, Spector, Sjoerdsma & Udenfriend, 1966; Alouisi & Weiner, 1966; Sedvall & Kopin, 1967; Roth, Stjärne & von Euler, 1967; Dairman, Gordon, Spector, Sjoerdsma & Udenfriend, 1968; Dairman & Udenfriend, 1970a). These effects may be a result of end product inhibition by catecholamines (Spector, Gordon, Sjoerdsma & Udenfriend, 1967; Neff & Costa, 1966). When animals are subjected to procedures which cause a sustained increase in sympathetic nerve activity, an apparent induction of tyrosine hydroxylase occurs (Mueller, Thoenen & Axelrod, 1969; Thoenen, Mueller & Axelrod, 1969; Viveros, Arqueros, Connett & Kirshner, 1969; Weiner & Mosimann, 1970; Kvetňanský, Gewirtz, Weise & Kopin, 1970; Patrick & Kirshner, 1971) which results in an accelerated rate of catecholamine formation (Dairman & Udenfriend, 1970b). In addition, the apparent activity of tyrosine hydroxylase in several tissues can be decreased after treatment with L-3, 4,dihydroxyphenylalanine (Dairman & Udenfriend, 1971; Tarver, Berkowitz & Spector, 1971). These factors may independently or in concert influence the *in vivo* activity of tyrosine hydroxylase and thus serve to regulate the rate at which catecholamines will be synthesized.

Recently, Shiman, Akino & Kaufman (1971) have reported that the activity of bovine adrenal tyrosine hydroxylase is subject to substrate inhibition by tyrosine when the putative natural cofactor, tetrahydrobiopterine is used. These workers have made the suggestion that variations in the endogenous concentrations of tyrosine could act as another mechanism for regulating the biosynthesis of catecholamines. The results of experiments designed to test this hypothesis *in vivo* are presented in this report.

**Materials and Methods.** — Female Sprague Dawley rats weighing 190-210 g were used. L-Tyrosine was purchased from Mann Research Laboratories and cycloheximide from Sigma Chemical Co. Rats were fasted for 16 h before killing in order to minimize variations in the endogenous concentrations of tyrosine. L-Tyrosine was administered intraperitoneally as a fine suspension in 0.9% w/v sodium chloride solution. Tyrosine concentrations were determined in tissues and plasma by the method of Waalkes & Udenfriend (1957). Noradrenaline and dopamine were extracted and assayed as previously described (Crout, 1961; Drujan, Sourkes, Layne & Murphy, 1959). Recoveries ranged from 70% to 90% and were corrected to 100% by means of an internal [$^{14}$C] standard. Those animals kept at 4° C were housed in individual metal cages in order to prevent conservation of heat due to huddling.

The data were analysed by Student's *t* test.

**Results.**—There are several ways by which the plasma and tissue concentrations of L-tyrosine can be increased. One method is to inject large amounts of the amino-acid itself (Chirigos, Greengard & Udenfriend, 1960). A second approach is to inhibit protein synthesis (Dairman & Udenfriend, unpublished observations).

TABLE 1. *Catecholamine concentrations after increases in tissue and plasma tyrosine concentrations produced by cycloheximide or* L-*tyrosine*

| | Tyrosine | | Noradrenaline | | Dopamine |
|---|---|---|---|---|---|
| | Brain ($\mu$g/g) | Plasma ($\mu$g/ml) | Brain ($\mu$g/g) | Heart ($\mu$g/g) | Brain ($\mu$g/g) |
| Exp. I | | | | | |
| Control | 14·2±0·5 | 14·6±1·7 | 0·52±0·01 | 1·40±0·08 | |
| L-tyrosine | 26·7±2·0‡ | 27·2±2·6‡ | 0·56±0·3 | 1·48±0·1 | |
| Exp. II | | | | | |
| Control | — | 13·4±1·64 | 0·39±0·01 | 1·03±0·05 | — |
| Cycloheximide 2·5 mg/kg | — | 23·4±2·78* | 0·44±0·04 | 1·19±0·25 | — |
| Exp. III | | | | | |
| Control | 13·5±2·3 | 11·8±1·0 | — | 0·96±0·09 | 0·93±0·1 |
| Cycloheximide 5·0 mg/kg | 60·7±1·5† | 88·1±4·5† | — | 1·19±0·14 | 0·80±0·08 |

Exp. I—Rats were given either 0·9% NaCl or 1,000 mg/kg of L-tyrosine intraperitoneally and placed in the cold at 4° C. Two and one half hours later the drug treatment was repeated. The animals were killed 3 h later.

Exp. II—Rats were injected intraperitoneally with either 0·9% NaCl or cycloheximide (2·5 mg/kg) and killed 6·5 h later. Five animals were used per group.

Exp. III—Rats were injected intraperitoneally with either 0·9% NaCl or cycloheximide (5 mg/kg) and killed 4·5 h later. Four animals were used in each group.

* $P<0·02$; † $P<0·001$; ‡ $P<0·005$. Student's $t$ test.

In the first experiment rats were placed in the cold at 4° C in order to increase their rate of catecholamine synthesis (Gordon *et al.*, 1966; Oliverio & Stjärne, 1965). One group of these animals received two doses of 1,000 mg/kg of L-tyrosine with a time interval of 2·5 h, which resulted in a 2-fold increase in the plasma and brain concentration of the amino-acid (Table 1). As is also shown in Table 1 the noradrenaline concentration in both the heart and the brain remained unchanged after this treatment.

It is possible to achieve high endogenous concentrations of tyrosine by inhibiting protein synthesis with cycloheximide. As shown in Table 1 the administration of cycloheximide resulted in as much as a 7-fold increase in both the brain and plasma concentrations of the amino-acid. Despite these high concentrations of tyrosine no reduction in the content of brain and heart catecholamines could be demonstrated.

**Discussion.**—Shiman *et al.* (1971) have shown that bovine adrenal tyrosine hydroxylase activity, when assayed using tetrahydrobiopterin as the pteridine co-factor, is maximal when the tyrosine concentration is in the order of $2\times10^{-5}$M. The tyrosine concentration in tissue and plasma of control rats was found to be $7·4\times10^{-5}$M. The substrate inhibition curve presented by Shiman *et al.* (1971) shows that this concentration should produce a 36% inhibition from the optimal rate. An increase in the tissue tyrosine concentration of 2-fold after the administration of tyrosine and 7-fold by cycloheximide treatment should result in a 46% and 65% inhibition, respectively, of the rate obtained in the presence of the control concentration of tyrosine. However, the results of our *in vivo* study do not confirm this expectation. If tyrosine hydroxylase was inhibited by the increased concentrations of tyrosine, then one should observe a decrease in the tissue catecholamine concentrations as is seen after the administration of the tyrosine hydroxylase inhibitor, $\alpha$-methyl-*p*-tyrosine (Spector, Sjoerdsma & Udenfriend, 1965). This would be a result of the inability of the synthesizing system to keep pace with catecholamine release and degradation (Gordon *et al.*, 1966). Even in those experiments in which the demand for catecholamines is greatly increased by exposure to cold, no diminution in the concentration of noradrenaline or dopamine was demonstrable when the tissue concentration of tyrosine was increased.

There are several possible explanations for the lack of agreement between the *in vivo* experiments presented in this report and the *in vitro* data (Shiman *et al.*, 1971). The ability of excess tyrosine to inhibit its own hydroxylation has been investigated only with respect to enzyme prepared

from beef adrenal. This phenomenon may be peculiar to this species or tissue. Also, this inhibition of tyrosine is not seen with all tetrahydropteridines (Shiman, *et al.*, 1971). Although there is evidence that tetrahydrobiopterin is the natural cofactor for tyrosine hydroxylase in beef adrenal (Lloyd & Weiner, 1970), the role of this pteridine in tyrosine hydroxylase activity has not yet been established for other species. A third possibility is that despite the increases in both tissue and plasma tyrosine concentrations, the concentration of this amino-acid remains unchanged within sympathetic and central nervous system neurones.

The experiments reported in this study were all done with the rat. There is good evidence that an increase in the tyrosine concentration in the tissues will not result in a physiological inhibition of tyrosine hydroxylase in at least one other species. Levitt *et al.* (1965), using the isolated guinea-pig heart, have shown that a maximum incorporation of [$C^{14}$]-L-tyrosine into noradrenaline occurred with a concentration of tyrosine in the perfusion fluid of approximately $8\times10^{-5}$M. Increasing the concentration of tyrosine in the perfusate up to almost $1\times10^{-3}$M did not result in any decrease in the rate of incorporation. These studies, since they were carried out on an isolated organ, eliminate the possibility of a compensatory increase in tyrosine hydroxylase activity mediated through sympathetic nerve activity or release from end product inhibition. The present studies do not support the concept that substrate inhibition of tyrosine hydroxylase by tyrosine can function as an *in vivo* mechanism for the regulation of catecholamine synthesis.

The author thanks Dr. S. Spector for helpful discussion and Miss G. Reed for technical assistance.

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(*Received September* 13, 1971)