# Estimates of catecholamine turnover rates in individual hypothalamic nuclei of the rat by use of alpha-methyl-para-tyrosine

J.S. KIZER, I.J. KOPIN & J.A. ZIVIN*
(introduced by J.L. REID)

*Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland, U.S.A.*

Administration of alpha-methyl-para-tyrosine (αMpT) or its methyl ester has been shown to inhibit tyrosine hydroxylase, the rate limiting step in catecholamine synthesis (Spector, Sjoerdsma, & Udenfriend, 1965). The disappearance of endogenous catecholamines after inhibition of tyrosine hydroxylase has been used to estimate the rate of turnover of catecholamines in brain and other tissues. However, there has been some debate concerning the proper method of administration and the validity of the estimates of turnover rates because the falls in catecholamine levels are multiphasic (Iversen & Glowinski, 1966; Costa & Neff, 1966; Javoy & Glowinski, 1971; Doteuchi, Wang & Costa, 1974).

The purpose of our study was to measure the turnover rates of catecholamines in various hypothalamic nuclei. The recent availability of microdissection techniques and highly sensitive biochemical methods for assay of biogenic amines has made it possible to localize more specifically alterations in the effects of catecholamine metabolism and thereby to define more precisely the functional significance of the different nuclei.

Adult, male rats (Zivic-Miller), 200-250 g were given various doses of L-αMpT or DL-αMpT methyl ester (Sigma), and killed by decapitation at intervals up to 5 h, after initial drug administration. The brains were rapidly removed, frozen, and microdissection was performed by the method of Palkovits, Brownstein, Saavedra & Axelrod (1974). Dopamine and other catecholamines (predominently norepinephrine) were then measured by a sensitive radiometric assay (Coyle & Henry, 1973).

Our findings were: (1) Intravenous administration of DL-αMpT methyl ester, 200 mg/kg, produced no inhibition of catecholamine synthesis. (2) After 200 mg/kg i.v. of L-αMpT, there was a biphasic decline in the dopamine and norepinephrine levels in the median eminence (ME), ventromedian nucleus (n v m), and medial forebrain bundle (MFB), with the more rapid decline occurring during the first hour. (3) L-αMpT, 200 mg/kg i.v., or DL-αMpT methyl ester, 400 mg/kg i.p., were inadequate to prevent levels of catecholamines from increasing after 3 h; therefore, a second dose was required at that time to maintain inhibition of tyrosine hydroxylase. (4) There appeared to be no significant difference in the turnover rates of catecholamines after administration of either L-αMpT, 200 mg/kg i.v., or DL-αMpT, 400 mg/kg i.p. (5) After 400 mg/kg DL-αMpT methyl ester i.p. at 0 and 3 h, the rate constants for the 1-5 h interval for dopamine in ME, n v m, and MFB were respectively: $0.154 \pm 0.058$, $0.176 \pm 0.068$, and $0.0964 \pm 0.066$ ($h^{-1}$, mean ± standard deviation, n = 10); and for norepinephrine for these same regions: $0.0633 \pm 0.0631$, $0.138 \pm 0.089$, and $0.142 \pm 0.056$.

The results of this study suggest that: (1) it is possible to estimate relative rates of turnover of catecholamines in small discrete areas of brain by inhibition of their synthesis; (2) large and frequent doses of the inhibitory agent must be administered to ensure effective blockade of synthesis; (3) because of the multiphasic nature of the decline in amine content, caution must be exercised in interpreting these results in absolute terms. This method may be of value, however, in identifying changes in catecholamine turnover and, by implication, functional activity of such nuclei after various forcings.

## References

COSTA, E. & NEFF, N. (1966). Isotopic and non-isotopic measurements of the rate of catecholamine biosynthesis. In *Biochemistry and Pharmacology of the Basal Ganglia*, (eds), Costa, E., Cote, L.J. & Yahr, M.D., pp. 141-155, Raven Press: New York.

COYLE, J.T. & HENRY, D. (1973). Catecholamines in fetal and newborn rat brain. *J. Neurochem.*, **21**, 61-67.

DOTEUCHI, M., WANG, C. & COSTA, E. (1974). Compartmentation of dopamine in rat striatum. *Molec. Pharmac.*, **10**, 225-234.

JAVOY, F. & GLOWINSKI, J. (1971). Dynamic characteristics of the functional compartment of dopamine in dopaminergic terminals of the rat striatum. *J. Neurochem.*, **18**, 1305-1311.

IVERSEN, L.L. & GLOWINSKI, J. (1966). Regional studies of catecholamines in the rat brain–II. *J. Neurochem.*, **13**, 671-681.

PALKOVITS, M., BROWNSTEIN, M., SAAVEDRA, J.M. & AXELROD, J. (1974). Norepinephrine and dopamine content of hypothalamic nuclei of the rat. *Brain Res.*, **77**, 137-149.

SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1965). Blockade of endogenous norepinephrine synthesis by α-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. *J. Pharmac. exp. Ther.*, **147**, 86-95.