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L-Tyrosine enhancement of the elevation of 3,4-dihydroxyphenylacetic acid concentration in rat brain by spiperone and amfonelic acid

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Several reports have indicated that administration of L-tyrosine to rats can increase dopamine synthesis and turnover in the brain in some but not all conditions (Scally et al 1977; Carlsson & Lindqvist 1978; Sved et al 1979; Melamed et al 1980; Sved & Fernstrom 1981). Apparently tyrosine availability can be rate-limiting to dopamine synthesis when dopamine turnover has been accelerated by pharmacological alteration of dopaminergic pathways.

Amfonelic acid and certain other dopamine uptake inhibitors cause marked further enhancement of dopamine synthesis when dopamine neuronal firing and dopamine synthesis are elevated by dopamine receptor blockers (Shore 1976; McMillen et al 1980). This enhancement may occur because dopamine reuptake is a feedback mechanism limiting dopamine synthesis (Cerrito & Raiteri 1981) or because amfonelic acid and related drugs facilitate the impulse-mediated release of dopamine by enhancing the mobilization of intraneuronal storage pools of dopamine (Shore et al 1979). Under these conditions when dopamine synthesis is increased by the combination of a dopamine antagonist and a dopamine uptake inhibitor, there is a several-fold rise in the concentration of the dopamine metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) (Shore 1976; Fuller & Snoddy 1979). The possibility that tyrosine availability limits dopamine synthesis in this situation and that tyrosine injection would further enhance the increase in DOPAC was the subject of this study.

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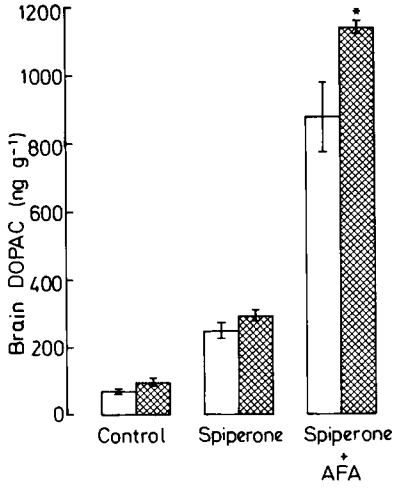

FIG. 1. DOPAC concentration in whole brain. Values for control rats, rats treated with spiperone, and rats treated with the combination fo spiperone and amfonelic acid (AFA) with (shaded bars) or without (open bars) injection of L-tyrosine 1 h before they were killed are shown as mean ± standard errors for 5 rats per group. * indicates significant effect ($P < 0.025$) of tyrosine injection.

Male Wistar rats (130–150 g) from Harlan Industries, Cumberland, Indiana, were housed in groups of 5 in hanging wire cages with free access to food and water in a 24 °C room with lights on from 0700–1900 h. L-Tyrosine (Calbiochem) was injected at 200 mg kg^{-1} i.p. 1 h before the rats were killed. Spiperone (Janssen) was injected at 0·5 mg kg^{-1} i.p. 2 h before rats were killed. Amfonelic acid (Winthrop) was injected at 5 mg kg^{-1} 1 h before rats were killed. The rats were decapitated, and whole brains were quickly removed, frozen on dry ice, and stored at −15 °C before analysis. DOPAC concentration was determined spectrofluorometrically by a modification (Fuller & Perry 1978) of the methods of Murphy et al (1969) and Spano & Neff (1971). Data are expressed as mean values ± standard errors for 5 rats per group. Comparisons between groups with/without tyrosine injection were made by Student's *t*-test.

Fig. 1 shows the concentration of DOPAC in rat brain 1 h after the injection of L-tyrosine into control rats or rats treated with spiperone alone or in combination with amfonelic acid. Injection of L-tyrosine did not cause a significant change in DOPAC concentration in control rats or in rats treated with spiperone, the latter rats showing 3·6-fold elevation of brain DOPAC due to spiperone. The combination of amfonelic acid plus spiperone produced a marked further increase in brain DOPAC to levels 3·5 times those in rats that received only spiperone. In these rats treated with spiperone and amfonelic acid, L-tyrosine injection significantly ($P < 0.025$) increased the concentration of DOPAC, the difference between the two means corresponding to 261 ng DOPAC g^{-1} brain. The magnitude of that change is greater than that produced by spiperone alone.

These results add an additional experimental paradigm to those in which the injection of L-tyrosine has been found to increase dopamine synthesis and turnover. Previous conditions in which such an effect of L-tyrosine has been noted were in rats treated with haloperidol (Scally et al 1977; Carlsson & Lindqvist 1978), reserpine (Sved et al 1979), or γ-butyrolactone (Sved & Fernstrom 1981), or in rats after partial lesioning of the nigrostriatal tract with

6-hydroxydopamine (Melamed et al 1980). The explanation has been proposed that when tyrosine hydroxylase is activated, its affinity for the cofactor, tetrahydrobiopterin, is increased so that the enzyme is no longer limited by tetrahydrobiopterin concentration but instead becomes limited by L-tyrosine availability (Sved & Fernstrom 1981). In these circumstances, exogenous tyrosine can increase dopamine synthesis. Our findings extend the list of pharmacological models in which this effect of tyrosine has been demonstrated.

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Suppression of ethanol consumption by MET-enkephalin in rats

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Recent experimental studies on the interactions between opiates and ethanol have been focused on the ability of the acute effects of opiate agonists to reduce the volitional consumption of ethanol in rats (Sinclair et al 1973; Ho et al 1976, 1980) and in hamsters (Ross et al 1976) whereas ethanol consumption is increased following opiate withdrawal (Ho 1980). With the identification of the opiate receptors and the detection and characterization of the endogenous opiate-like peptides, the enkephalins and endorphins (Hughes et al 1975; Goldstein 1973), it is reasonable to suspect that some of these endogenous opioids may alter ethanol consumption in a way similar to the opiates. We now present some preliminary data to show that met-enkephalin injected into the rat lateral ventricle under conscious states, significantly suppressed the volitional consumption of ethanol and the effect was partially reversed by naltrexone.

Adult male Long-Evans hooded rats, 200 to 250 g, were kept in individual cages at a constant temperature (20 °C) and humidified room. Two graduated glass drinking tubes (Richter type, Kimax Instrument Co.) were fitted on to the outside of each cage, one filled with water and the other with 5% v/v ethanol (diluted from 95% ethanol with deionized water). The rats were put on a training schedule and were allowed 2 h (from 1 to 3 p.m.) for fluids (water and ethanol) and food ingestion each day. After a stable base-line of consumption was established, the animals were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.) supplemented by ether during surgery when necessary and were stereotactically implanted in the horn of the lateral ventricle with permanent hollow stainless steel cannulae 5 mm long. Coordinates employed were from bregma 0·6 P., 1·8 L and from the surface of the brain 3·1 mm. A minimum of 7 days postoperative recuperation was allowed before they were put back on a food and water/ethanol training schedule. After a stabilizing period to re-establish a base-line of consumption the rats ($n = 8$) were injected under conscious conditions with 20 μl with a vehicle solution of either artificial cerebrospinal fluid (c.s.f.) or 0·9% NaCl 30 min before the food and water/ethanol session. The administrations of the vehicle solution were repeated several times so as to avoid the initial stress induced by the treatment procedure. Met-enkephalin (supplied by Peninsula Laboratory, California) was dissolved in 20 μl artificial c.s.f. at three different concentrations (40, 80 and 200 μg) and injected under conscious states into the ventricle. Fig. 1 shows that following a met-enkephalin in 200 μg injection, a significant ($P < 0{\cdot}005$) reduction was observed in ethanol consumption which lasted for at least 2 days. At the lower concentrations of 40 and 80 μg/rat, there was only a small and transient reduction in ethanol intake. The rats appeared to be normal in their gross behaviour and in good general condition following an intraventricular injection; there was no significant loss in weight. The lack of response at the lower dose levels may be attributed to the rapid inactivation of met-enkephalin by peptidases in the c.n.s. and thus reducing its availability at the opiate receptors. No significant reduction in ethanol consumption was observed in the 'sham' operated controls given equal

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