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Tyrosine: Effects on Catecholamine Release

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ACWORTH, I. N., M. J. DURING AND R. J. WURTMAN. *Tyrosine: Effects on catecholamine release.* BRAIN RES BULL 21(3) 473–477, 1988.—Tyrosine administration elevates striatal levels of dopamine metabolites in animals given treatments that accelerate nigrostriatal firing, but not in untreated rats. We examined the possibility that the amino acid might actually enhance dopamine release in untreated animals, but that the technique of measuring striatal dopamine metabolism was too insensitive to demonstrate such an effect. Dopamine release was assessed directly, using brain microdialysis of striatal extracellular fluid. Tyrosine administration (50–200 mg/kg IP) did indeed cause a dose related increase in extracellular fluid dopamine levels with minor elevations in levels of DOPAC and HVA, its major metabolites, which were not dose-related. The rise in dopamine was short-lived, suggesting that receptor-mediated feedback mechanisms responded to the increased dopamine release by diminishing neuronal firing or sensitivity to tyrosine. These observations indicate that measurement of changes in striatal DOPAC and HVA, if negative, need not rule out increases in nigrostriatal dopamine release.

Dopamine Tyrosine Striatum Extracellular fluid Neurotransmitter-release Microdialysis

EXISTING data suggest that the relationship between the tyrosine level in a catecholaminergic neuron and the rate at which the amino acid is converted to dopamine (or norepinephrine or epinephrine) is complex (31). Tyrosine given systemically increases catecholamine synthesis and release, as estimated by measuring brain levels of various catecholamine metabolites, within neurons that have been made to fire frequently (7, 14, 15, 26). But the amino acid seems to have no effect on neurons that have not been pretreated so as to render them especially active (6, 7, 23, 26, 32, 33).

Thus, for example, if rats receive partial unilateral nigrostriatal lesions (which accelerate the firing of the surviving dopaminergic neurons in that hemisphere), tyrosine administration increases the levels of dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) per surviving neuron *ipsilateral* to the partial lesion, but has no effect on dopamine metabolite levels in the intact, contralateral side of the brain (23). Similar responsiveness to supplemental tyrosine, as estimated by measuring brain levels of DOPAC and HVA, or of the norepinephrine metabolite, 3-methoxy-4-hydroxy-phenylglycol sulfate (MHPG-sulfate), can be generated by giving rats drugs which impair catecholaminergic neurotransmission [e.g., reserpine (33); haloperidol (7,26)], of physiological treatments which selectively activate particular catecholaminergic tracts [like prolactin administration (30) or stress (15,20)].

This correlation between the tyrosine-responsiveness of a neuron and its apparent firing frequency is compatable with evidence that neuronal firing activates the enzyme tyrosine hydroxylase [by allosteric modification (11,36)]. This activation increases the enzyme's affinity for its cofactor tetrahydrobiopterin (21), diminishing its sensitivity to end-product inhibition (2), and makes the enzyme's net activity depend on its saturation with tyrosine. As might be expected tyrosine does increase dopamine synthesis in otherwise untreated animals within neurons with high spontaneous firing frequencies and bursting activities (4), [such as the mesocortical dopamine neurons (34)]; the tyrosine hydroxylase in such neurons is thought to be tonically activated.

Assuming that at least *some* of the enzyme in the nigrostriatal neurons of untreated animals would be in the activated state, it seems surprising that in this circumstance, tyrosine had no apparent effect on dopamine production or release. We wondered whether this lack of effect simply reflected the use of too insensitive an experimental system. Hence we have used a newer approach to study tyrosine's effects, that of directly assessing dopamine levels within the intrastriatal extracellular fluid (ECF) obtained by intracerebral dialysis. This method is increasingly becoming established as a useful technique for obtaining repeated samples of brain ECF in vivo. The ECF is presumably in equilibrium with the contents of local synapses, allowing measure of released dopamine (9, 18, 19, 28, 35, 37) and also of other monoamine neurotransmitters (1, 3, 27, 29). Our data show that tyrosine administration does indeed increase the dopamine concentration in striatal ECF of otherwise-untreated animals, but that this increase is transient, probably because it effectively enhances the occupancy of intrasynaptic dopamine receptors (5), slowing neuronal firing and activating autoreceptor-mediated decreases in dopamine release. Levels of the dopamine metabolites DOPAC and HVA in striatal ECF or whole brain do not rise in parallel after tyrosine administration, with those of ECF dopamine, hence negative data from previous experiments which estimated dopamine release by measuring these metabolites in brain, should be interpreted with caution.

[1]This paper is derived from a talk given by D. Richard Wurtman to The Department of The Air Force, held in San Diego in 198[illegible]

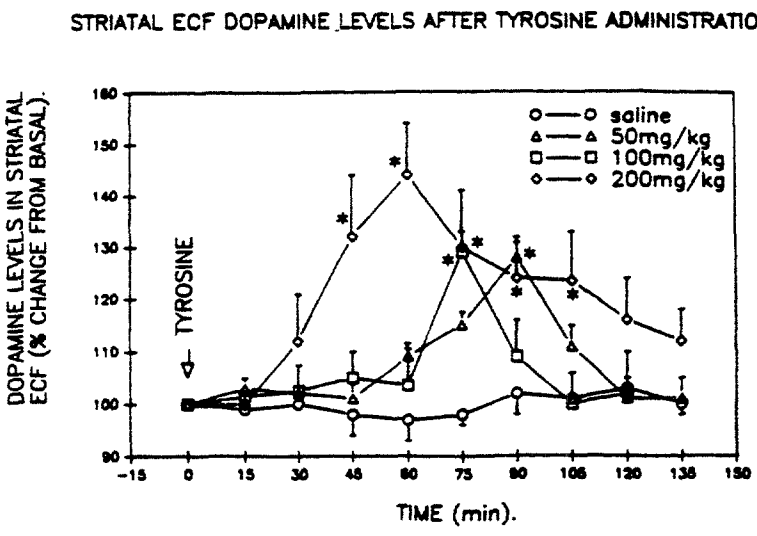

FIG. 1. The effect of intraperitoneal administration of saline or various doses of tyrosine on striatal ECF levels of dopamine. Rats were anaesthetized with alpha-chloralose/urethane (50/500 mg/kg IP). Probes were placed acutely in the right striatum (A +0.5, R 2.5, V −7) and were perfused with artificial cerebrospinal fluid at 1.5 μl/min. After injury release (100–120 min postprobe implantation), and when the basal levels of dopamine in three consecutive samples varied by less than 8%, rats then either received saline or tyrosine (50, 100 or 200 mg/kg, 4 ml/kg) as a suspension (in physiological saline) intraperitoneally. Samples were analyzed every 15 min for dopamine by HPLC-electrochemical detection. Groups of five animals were used for each drug treatment. Vertical bars represent standard error of the mean. Statistical significance was measured using Student's *t*-test (unpaired). $^*p<0.05$.

METHOD

Animal Preparation

Male Sprague-Dawley rats (300–400 g) were anaesthetized with alpha-chloralose/urethane [50/500 mg/kg intraperitoneally (IP)] and placed in a Kopf stereotaxic frame. The skull was exposed and a hole drilled through the cranium above the right striatum using the following coordinates: A +0.5, R 2.5, V −7 (dura) taken from Paxinos and Watson (24). The position of the probe was verified by measuring the basal concentration of dopamine in the ECF and also after post-mortem sectioning. Microdialysis probes, 0.5 mm outer diameter, 4 mm exposed membrane length and 5000 Dalton "cut-off" (Carnegie Medicin, Solna, Sweden) were implanted into the striatum, 7 mm from the dura, (the membrane spanned the striatum −3 to −7 mm from the dura). The dialysis probes were perfused with an artificial cerebrospinal fluid (Na^+ 147 mM, K^+ 3.5 mM, Ca^{2+} 1.0 mM, Mg^{2+} 1.2 mM, Cl^- 129 mM, phosphate 1 mM, HCO_3^- 25 mM, CO_2/O_2 to pH 7.4) at 1.5μl/min, using a CMA microperfusion pump (Carnegie Medicin, Solna, Sweden). Animals were maintained at a stable level of anaesthesia by administration of additional doses of anaesthetic if required. The body temperature was monitored using a colonic probe and maintained at 37°C using a heating pad.

Samples were collected every 15 min into 5.0 μl of 0.5 M perchloric acid to minimize air oxidation of dopamine. Following probe implantation, the first 100–120 minute collections were discarded after assay of a few had confirmed increased dopamine concentrations due to "injury" release (18, 19, 35), probably resulting from neuronal damage during probe placement. Samples were then analyzed for measurement of baseline dopamine release after three consecutive ones had dopamine concentrations varying by less than 8%. Rats then either received physiological saline or drug treatment (amino acids were given as a suspension (in physiological saline), 4 ml/kg IP).

HPLC Analysis

The liquid chromatographic system consisted of an Altex pump with dual, in-series SSI suppresors; a Rheodyne switching valve with 20 μl loop; a 3 μ HR-80 C18 column (ESA Inc. Bedford, MA); and ESA 5100A coulometric detector with in-line conditioning cell. In-line filters were placed postRheodyne and postcolumn. The mobile phase consisted of 70 mM sodium phosphate, 0.63 mM heptane sulphonic acid and 0.22 mM EDTA, pH 4.2: methanol 5% v/v. The flow rate was 1.8 ml/min. Chromatograms (DOPAC, dopamine, 5HIAA, HVA and 5HT) were completed within 12 minutes thereby allowing immediate on-line analysis.

Recoveries

Daily recoveries by each probe were initially measured both before implantation and after removal from the brain at the end of the experiment. Probes were calibrated (at a flow rate of 1.5 μl/min) in vivo by placing them in 0.1 μM standards, at *room temperature*, and were collected in perchloric acid as above. After several minutes (to allow development of a steady-state across the probe's membrane, of com-

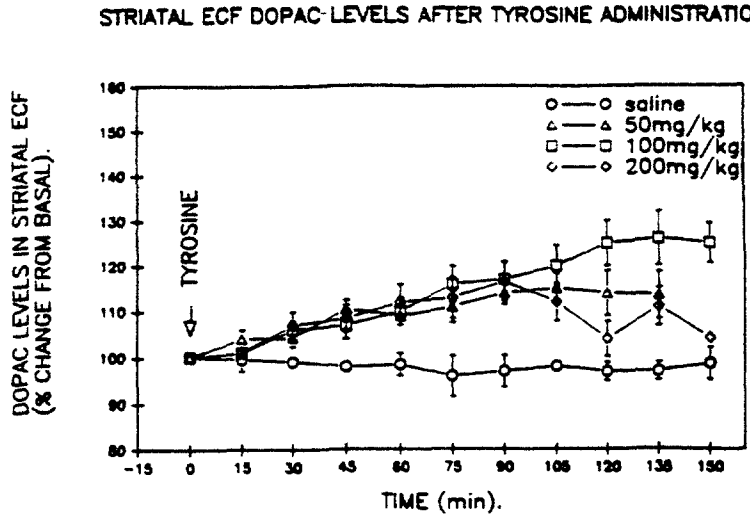

FIG. 2. The effect of intraperitoneal administration of saline or various doses of tyrosine on striatal ECF levels of DOPAC. Animals were prepared as described in the legend to Fig. 1. Samples were collected every 15 min and were analyzed for DOPAC using HPLC-electrochemical detection. Groups of five animals were used for each drug treatment. Vertical bars represent standard error of the mean.

pounds to be measured) the concentrations of standards were measured in two or three fractions. Under these conditions (i.e., at the temperature and flow rate indicated) the Carnegie Medicin probes exhibited little between-probe variability, and the percentage recoveries of dopamine, DOPAC and HVA were 16%, 26% and 31% respectively. There was little variability in recoveries, before or after implantation. Recoveries were only used to allow between-animal comparison. They are not used to give "calculated" extracellular fluid concentrations: this could be misleading, since the diffusion kinetics in vivo may differ from those in vitro (16).

Statistics

Results were given as mean±standard error of the mean. Statistical analysis of the data was determined using Student's *t*-test (unpaired).

RESULTS

Under basal conditions, the extracelluar concentration of dopamine was 4.62±0.48 nM (n=15), that of DOPAC was 2092±148 nM and that of HVA was 1388±77 nM. These levels are in agreement with values reported previously (8, 18, 19, 35, 37).

The effects of saline or tyrosine (50, 100 and 200 mg/kg IP) (n=5 per group) on the concentration of dopamine in striatal extracellular fluid are shown in Fig. 1. The two lower doses of tyrosine transiently and significantly ($p<0.05$) increased the extracellular concentration of striatal dopamine by 28±4% at 90 min and 29±4% at 75 min for the 50 and 100 mg/kg doses respectively. The peak increase in ECF dopamine (45±9%; $p<0.05$) occurred 60 min after rats received the highest (200 mg/kg) tyrosine dose. The ECF DOPAC and HVA levels showed a slight tendency to increase with all doses of tyrosine, but these increases were not dose-related (Figs. 2 and 3).

In related studies, using the same experimental system reported above, we have examined the effect of haloperidol (2 mg/kg IP) pretreatment (90 min before saline or tyrosine) on ECF levels of dopamine and its metabolites in control animals and those receiving tyrosine (100 mg/kg IP). The striatal ECF levels of dopamine, DOPAC and HVA are elevated but stable (for at least 140 min) ninety minutes after animals received haloperidol (2 mg/kg IP). After haloperidol pretreatment the increase in striatal ECF dopamine levels caused by supplemental tyrosine are maintained up to 146±8% for at least 180 min (in preparation).

DISCUSSION

These data support the following conclusions: 1) Supplemental tyrosine—given in doses which need not elevate plasma tyrosine beyond its physiological range (12, 13, 22)—can indeed enhance the release of dopamine from nigrostriatal neurons in otherwise-untreated animals. But, 2) This effect is transient, probably because it triggers receptor-mediated feedback mechanisms that slow neuronal firing (5), and/or act presynaptically to diminish transmitter release per firing (5). These hypothetical mechanisms would be expected to bring *net* dopamine release per neuron, per unit time, back to levels existing before the amino acid was given. (In agreement with this explanation, treatment with a drug—haloperidol—that interrupts these feedback mechanisms allowed tyrosine to produce continuing increases in dopamine release.) And, 3) Significant increases in the release of dopamine from nigrostriatal terminals need not be associated with parallel increases in ECF or whole-brain levels of dopamine's chief metabolites. DOPAC and HVA. A similar dissociation between ECF levels of dopamine and its metabolites has also been observed in animals given various drugs (18, 19, 35). The dissociation that we observed after tyrosine probably reflects the low concentration of

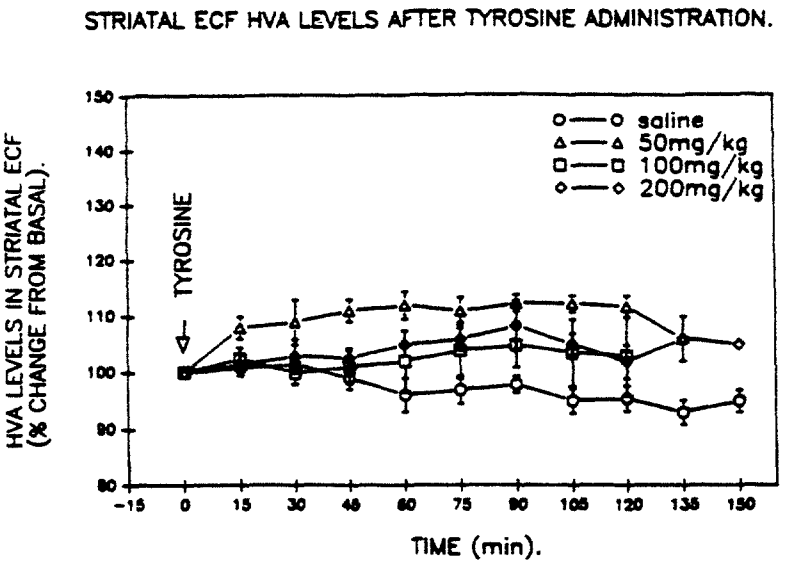

FIG. 3. The effect of intraperitoneal administration of saline or various doses of tyrosine on striatal ECF levels of HVA. Animals were prepared as described in the legend to Fig. 1. Samples were collected every 15 min and were analyzed for HVA using HPLC-electrochemical detection. Groups of five animals were used for each drug treatment. Vertical bars represent standard error of the mean.

dopamine in striatal ECF, relative to those of DOPAC and HVA, as well as the likelihood that a high proportion of the metabolite molecules may be formed within the nerve terminal from dopamine destroyed locally without ever having been released into the synaptic cleft.

We have also observed that tyrosine is not the only naturally occuring amino acid capable of altering ECF dopamine concentrations. Phenylalanine, depending on the dose given, can increase dopamine levels, not alter them, or even decrease them (9,10). The apparent reason for this complex effects is that low phenylalanine doses are rapidly and quantitatively converted to tyrosine by the animal's liver, but high doses elevate blood and brain phenylalanine by more than they elevate tyrosine, and phenylalanine both competes with tyrosine for brain uptake (25) and directly inhibits tyrosine hydroxylase activity (17). Increases or decreases in ECF dopamine levels produced by various phenylalanine doses were not accompanied by parallel changes in the ECF concentration of DOPAC or HVA. Another large neutral amino acid, valine (200 mg/kg IP), had no detectable effect on ECF dopamine levels (data not shown), indicating that the effects of tyrosine or phenylalanine are not simply stress responses.

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