Short communications

Effects of α-methyl-*p*-tyrosine and L-DOPA on brain self-stimulation and motor activity in rats

J. BEAUGRAND AND J. ST.-LAURENT

Département de Psychologie, Université du Quebec, Montréal and Département de Psychiatrie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Canada

Rats with electrodes implanted in the posterior lateral hypothalamus and ventromedial tegmentum were trained to self-stimulate. Animals were treated (i.p.) first with α-methyl-*p*-tyrosine (α-MPT) and then L-DOPA; their self-stimulation rates and spontaneous behaviour were recorded. α-MPT (100 mg/kg) had an immediate and long lasting suppressive effect on self-stimulation, within the first half hour. L-DOPA administration failed to reinstate self-stimulation. Hypoactivity also followed α-MPT injections. While the behavioural changes were minimal after L-DOPA 50 mg/kg, a decrease of spontaneous activity and moderate hyper-reactivity were observed following L-DOPA at a dose of 200 mg/kg. Theoretical implications are discussed.

α-Methyl-*p*-tyrosine (α-MPT), a potent inhibitor of tyrosine hydroxylase, when injected into self-stimulating animals produces a deficit in brain catecholamines concomitant with a reduction in the rate of self-stimulation obtained with electrodes implanted in brain regions corresponding to the medial-forebrain-bundle (Poschel & Ninteman, 1966; Gibson, McGeer & McGeer, 1970; Cooper, Black & Paolino, 1971). Since α-MPT affects noradrenaline as well as dopamine (Spector, Sjoerdsma & Udenfriend, 1965; Weissman & Koe, 1965), it is not clear which amine is critical for self-stimulation. The present experiments were undertaken in order to determine whether an increase in the brain dopamine concentration produced by L-3,4-dihydroxyphenylalanine (L-DOPA) injection could reverse the suppressive effects of a previous α-MPT administration on self-stimulation and associated spontaneous behaviour.

Methods.—The experiments were carried out on 8 adult albino rats (300–350 g). One bipolar electrode (twisted stainless-steel wires, 250 μm in diameter and insulated except for the cross section of the tips) was implanted in each animal. Surgery was performed under sodium pentobarbitone anaesthesia, 50 mg/kg i.p. The electrodes were aimed at the posterior medial-forebrain-bundle at coordinates, 5 mm posterior to bregma, 1·5 mm lateral to the sagittal suture, and 8 mm down from the top of the skull. After a 10 day recovery period, the rats were trained to depress a metal lever which delivered a 0·25 s train of sine waves of 60 Hz to the brain *via* the implanted electrode. A 60 μA (peak to peak) current was used throughout the study. The animals were given a half hour self-stimulation session daily for at least 20 days, until their performance showed good stability. On the first day of the experiment, a 10 min reading of self-stimulation was taken and the rats were injected i.p. with α-MPT, 100 mg/kg at 0 h 15 min and 3 h 15 min. Readings were taken for 10 min at each subsequent half hour. Each reading period was initiated by 3 intracranial shocks used for re-adjusting the individual current to the original 60 μA. There were 6 readings following the first and second α-MPT injections. The last reading was followed by 12 h rest and 5 additional readings were taken. L-DOPA was then administered i.p. at 20 h 15 min (50 mg/kg for the first injection) and at 21 h 15 min (200 mg/kg for the second injection). Two readings of self-stimulation were taken between the L-DOPA injections, and 4 following the last injection. After 2 days' rest, post-experiment control readings were taken for the 4 following days. After a further 5 days of recuperation with 30 min of self-stimulation daily, the animals were submitted to the same procedure used in the above drug study but with placebo injections consisting of the vehicles in which the drugs were administered. Drugs used were DL-α-methyl-*p*-tyrosine and L-DOPA (L-3,4-dihydroxyphenylalanine). Solutions were prepared by mixing each drug with an equivalent amount of gum arabic and suspending in 0·9% w/v NaCl solution by homogenization. The placebo was always a mixture of gum arabic and 0·9% NaCl solution. The total volume of each injection was about 0·8 ml. Upon completion of the experiment, the rats were killed with pentobarbitone sodium (200 mg/kg). The brains were fixed in

formaldehyde (10%), transverse sections were made and stained according to the luxolfast blue technique of Klüver & Barrera (1953) for histological verification of electrode positions.

Results

Histology. The 8 electrodes were located either in the dorsolateral hypothalamus at the level of the mamillary bodies or in the ventromedial tegmentum both in or around the medial-forebrain-bundle and the area of Tsai.

Self-stimulation

*Effect of α-methyl-*p*-tyrosine.* A significant decrease in self-stimulation was noted following the first injection of α-MPT, 100 mg/kg, within the first half hour (t=5·729, P<0·01 Fisher's test). After 2 h, self-stimulation was almost completely abolished (Fig. 1). The injection of α-MPT, 100 mg/kg after 3 h had no further additional effects on self-stimulation. The control animals continued self-stimulating at baseline rates (Fig. 1). After 12 h, self-stimulation had increased significantly (t=3·160, P<0·05) when compared to the last readings of the previous day after injection of α-MPT. However, the rate reached less than half of that of the control group (t=4·789, P<0·01). By the 2nd reading, the rates had increased to about half of the value of the control group (t=3·319, P<0·02).

Effects of L-*DOPA* After the first injection of L-DOPA, 50 mg/kg, the self-stimulation decreased significantly within the first half-hour (t=2·476, P<0·05). In the following half hour, self-stimulation was re-established almost to the value reached in the tests before the injection of L-DOPA and no significant difference was observed (t=1·409, P>0·20). After the second injection of L-DOPA, 200 mg/kg, the same pattern of reduction and recovery of self-stimulation rates was observed; however, 1·5 h after the second injection, self-stimulation had started to decrease again (t=1·963, P>0·10) to become completely abolished 2 h after the second injection (t=2·476, P<0·05). After 2 days' rest, self-stimulation rates taken for 4 days (Fig. 1) reached the value attained before treatment with α-MPT and L-DOPA.

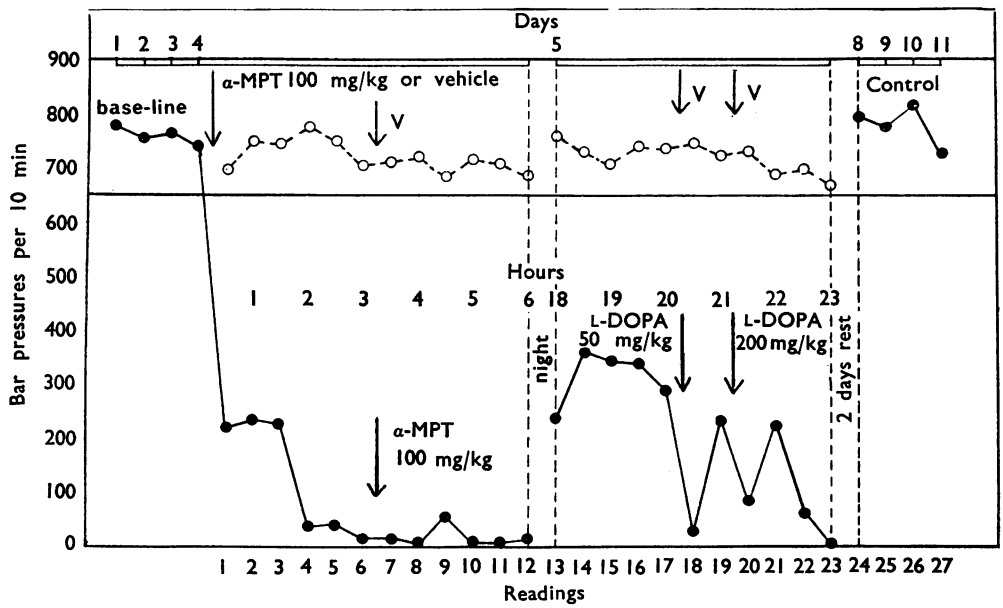

FIG. 1. Summary of the effects of α-methyl-*p*-tyrosine (α-MPT) and L-DOPA on self-stimulation rates. α-MPT, 100 mg/g, was injected at 0 h 15 min and 3 h 15 min. L-DOPA was administered to the rats at 20 h 15 min (50 mg/kg) and 21 h 15 min (200 mg/kg). The 4 baseline and 4 post-treatment control readings were taken daily for 10 min periods. On the experimental day, for both control (dashed line) and treated (continuous line) rats, all readings were taken for 10 min every half hour except for a 12 h rest period after the 12th reading.

Spontaneous behaviour

Effects of α-methyl-p-tyrosine. Before and after the injection of α-MPT, a brief neurological examination was made. It consisted of evaluating motor activity, then picking up the animal, looking for signs of hyper-reactivity such as startle response and aggressiveness. Finally, the righting reflex and equilibrium of the rat were tested. Fifteen minutes after the first injection, the rats showed drowsiness and adynamia i.e. a decrease of spontaneous movements with hypotonia. During this examination, the rats, easy to handle and not aggressive, had their righting reflex slightly slowed. However, their jumping activity and equilibrium appeared normal. There was no squealing and no retraction of the abdomen even when the animals were handled. There was no startle response to sudden noise (clap of hands). Electrical stimulation of the brain 15 and 25 min after injection of α-MPT elicited no behavioural changes. After 40 min, slight sniffing and exploration were elicited by electrical stimulation. After 80 min, the drowsiness and adynamia were less pronounced and 3 rats had started to self-stimulate again. After 170 min, the animals still showed less spontaneous movements but did not appear drowsy or show hypotonia and the remainder of the neurological examination was normal. Fifteen minutes after the second injection of α-MPT, all animals became hypoactive, drowsy, exhibited hypotonia and their righting reflex was slowed down. Most of these effects were still present 120 min after the second injection; only after 150 min did the animals become slightly more active with less hypotonia. After the 12 h rest, the behaviour was back to normal.

Effects of L-DOPA. Fifteen minutes after the injection of L-DOPA, 50 mg/kg, the animals showed less spontaneous movements but no hypotonia; no startle response and no aggressiveness were noted, however, the righting reflex was slightly slowed down. They showed some exploration during intracranial stimulation. Twenty minutes after the second injection of L-DOPA, 200 mg/kg, polypnoea was noted. In addition, two rats showed hypersalivation; one showed gnawing. There was a decrease of spontaneous activity, a slight increase of reactivity to noise and aggressiveness when handled. Righting reflex and jumping were normal. Electrical stimulation of the brain elicited some exploration and slight increase of motor activity, but there was no return to self-stimulation. After 50 min, hypoactivity was still present but there was no longer any startle response or aggressiveness.

Discussion.—Our results show reduced rates of self-stimulation following administration of α-MPT as observed in other studies (Poschel & Ninteman, 1966; Gibson *et al.*, 1970; Cooper *et al.*, 1971). In our study, the reduction of self-stimulation by α-MPT took place within the first half hour and so preceded the reduction of catecholamines which theoretically takes place later. In Poschel & Ninteman's (1966) study, the immediate suppression of self-stimulation with α-MPT (i.p.) was not mentioned. In the search for the mechanisms involved in the early effects of α-MPT, Moore (1966) reported that α-MPT injections appeared to be irritant as there was vocalization and retraction of the abdomen and some rats eventually died. Immediately following α-MPT injection, we did not observe retraction of the abdomen nor squealing and unlike Moore (1966) and Weissman & Koe (1965) we observed sedation as did van Rossum (1963). In addition, the animals which exhibited hypotonia and were tame, did not show any behavioural response to intracranial electrical stimulation. It appears that a toxic effect alone cannot explain the immediate suppressive effects on self-stimulation and spontaneous locomotor activity observed here, although such a factor cannot be excluded.

Injection of L-DOPA after α-MPT, did not reinstate self-stimulation but reduced it further and decreased spontaneous motor activity. L-DOPA did not reverse the effects of α-MPT, a result unlike those of Moore & Rech (1967) and Bédard, La Rochelle, Poirier & Sourkes (1970). Our results are more in accord with those of Boissier & Simon (1966) and Smith & Dews (1962), who found that in rats and mice, L-DOPA alone at doses of less than 500 mg/kg could suppress spontaneous motor activity and various types of operant behaviour. However, as observed by Boissier & Simon (1966), the diminution of motor activity was not accompanied by calmness; on the contrary, the animals were hyper-reactive. Hence, the decreased motor activity could be due to an exaggerated alertness leading to a 'freezing' effect.

Other studies suggest that central effects of L-DOPA are excitatory and the depressant effects on behaviour reported earlier were probably attributable to autonomic effects (Blaschko & Chrusciel, 1960; Smith & Dews, 1962). We observed tachypnoea and hypersalivation only after the second injection of L-DOPA at a dose of 200 mg/kg. Should noradrenaline be the main chemical mediator involved in self-stimulation, the administration of L-DOPA may not necessarily re-establish self-stimulation as L-DOPA would more readily restore the concentration of dopamine than that of noradrenaline in the brain (Carlsson, 1964; Bertler & Rosengren, 1959) and noradrenaline concentrations have been reported to decrease following L-DOPA treatment alone (Butcher & Engel, 1969).

This work was supported by the Medical Research Council of Canada, grant MA 3289 and by Establishment grant from the Conseil de la Recherche Médicale du Québec.

We are grateful to Enrique Ramon-Moliner for help in the localization of electrodes sites, L. J. Poirier and R. Leclerc for their criticisms and Marcel Paradis for skilful technical assistance.

REFERENCES

BÉDARD, P., LAROCHELLE, L., POIRIER, L. J. & SOURKES, T. L. (1970). Reversible effect of L-dopa on tremor and catatonia induced by α-methyl-*p*-tyrosine. *Can. J. Physiol. Pharmac.*, **48**, 82–84.

BERTLER, A. & ROSENGREN, E. (1959). On the distribution in brain monoamines and of enzymes responsible for their formation. *Experientia*, **15**, 382–384.

BLASCHKO, H. & CHRUSCIEL, T. (1960). The decarboxylation of amino acids related tyrosine and their awakening action in mice. *J. Physiol., Lond.*, **151**, 272–284.

BOISSIER, J. R. & SIMON, P. (1966). De la potentiation de la Dopa par les inhibiteurs de la monoamine oxydase. *Psychopharmacologia (Berl.)*, **8**, 428–436.

BUTCHER, L. L. & ENGEL, J. (1969). Behavioural and biochemical affects of L-dopa after peripheral decarboxylase inhibition. *Brain Res.*, **15**, 233–242.

CARLSSON, A. (1964). Functional significance of drug-induced changes in brain monoamine levels. *Progr. Brain Res.*, **8**, 9–27.

COOPER, B. R., BLACK, W. C. & PAOLINO, R. M. (1971). Decreased septal-forebrain and lateral hypothalamic reward after alpha-methyl-*p*-tyrosine. *Physiol. Behav.*, **6**, 425–429.

GIBSON, S., MCGEER, E. G. & MCGEER, P. L. (1970). Effect of selective inhibitors of tyrosine and tryptophan hydroxylases on self-stimulation in the rat. *Expt. Neurol.*, **27**, 283–290.

KLÜVER, H. & BARRERA, E. (1953). A method for the combined staining of cells and fibres in the nervous system. *J. Neuropath. exp. Neurol.*, **12**, 400–403.

MOORE, K. E. (1966). Effects of α-methyl-tyrosine on brain catecholamines and conditioned behaviour in guinea pigs. *Life Sci.*, **5**, 55–65.

MOORE, K. E. & RECH, R. H. (1967). Reversal of alpha-methyl-tyrosine induced behavioural depression with dihydroxyphenylalanine and amphetamine. *J. Pharm. Pharmac.*, **19**, 405–407.

POSCHEL, B. P. H. & NINTEMAN, F. W. (1966). Hypothalamic self-stimulation: its suppression by blockage of norepinephrine biosynthesis and reinstatement by metamphetamine. *Life Sci.*, **5**, 11–16.

ROSSUM, J. M. VAN (1963). Mechanism of the central stimulant action of α-methyl-metatyrosine. *Psychopharmacologia*, **4**, 271–280.

SMITH, C. B. & DEWS, P. B. (1962). Antagonism of locomotor suppressant effects of reserpine in mice. *Psychopharmacologia. (Berl.)*, **3**, 55–59.

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

WEISSMAN, A. & KOE, B. K. (1965). Behavioural effects of L-alpha-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. *Life Sci.*, **4**, 1037–1048.

(*Received August* 10, 1972)