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BRIEF COMMUNICATION

Effects of Apomorphine on Self-stimulation[1]

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ST-LAURENT, J., R. R. LECLERC, M. L. MITCHELL AND T. E. MILIARESSIS. *Effects of apomorphine on self-stimulation.* PHARMAC. BIOCHEM. BEHAV. 1(5) 581–585, 1973.–In rats, self-stimulation (SS) from posterior lateral hypothalamus and ventromedial tegmentum was suppressed by the ip administration of alpha-methyl-para-tyrosine methyl ester (α-MPT, 100 mg/kg). Apomorphine (0.25 or 0.5 mg/kg) was injected ip 3 1/2 hr after α-MPT treatment. Self-stimulation was reinstated to a significant degree, after 2 hr for the 0.25 mg/kg group, and 3 hr for the 0.5 mg/kg group. Apomorphine given after saline control, elicited an immediate suppression of SS for approximately 1/2 hr in the case of 0.25 mg/kg and 1 1/2 hr for 0.5 mg/kg.

Apomorphine Exploration Habituation Rat Activity

ALPHA-methyl-para-tyrosine methyl ester (α-MPT) is a potent inhibitor of tyrosine hydrosylase, thus limiting the synthesis of catecholamines such as dopamine (DA) and noradrenaline (NA) [16, 17, 20, 28, 33, 34]. Appropriate doses of α-MPT produce a deficit in brain catecholamines associated with the suppression of the self-stimulation (SS) performance normally obtained from brain regions corresponding to the medial forebrain bundle (MFB) [8, 13, 24]. In a previous study [3] we observed that L-Dopa (50 mg and 200 mg/kg) could not reinstate SS after it had been suppressed by α-MPT (100 mg/kg, ip). In addition, it was noted that α-MPT had an immediate suppressive effect on SS (within the first 30 min following injection), while L-Dopa gave hyperreactivity and decrease of spontaneous motor activity and SS. Weissman *et al.* [34] observed that when α-MPT was administered either 30 min after, or concomitant with amphetamine, the stereotyped symptoms were blocked in most of the rats. In addition to evidences of an early effect of α-MPT on amphetamine stereotyped symptoms, they observed that L-Dopa in high doses (320 mg/kg) could not reverse the effect of α-MPT.

Maj *et al.* [18] and Carlsson [6] obtained an increase of motor activity and of exploration respectively after administration of apomorphine while an increase of motor activity [26,31] and increased exploration [7, 14, 19, 30, 31] were reported to be the most significant behavioral features correlated with SS from the lateral hypothalamus and ventromedial tegmentum. It was felt that apomorphine could reinstate these behaviors and SS after their suppression by α-MPT pretreatment. In addition, α-MPT is not known to produce a blockage of dopaminergic receptors while apomorphine is said to be a direct stimulator of these receptors, which would account for the increase in motility [32] and in the stereotypies which it induced in rats. Hence, there appears to be a pharmacological basis for assuming that apomorphine would reinstate SS after its suppression by α-MPT.

METHOD

Animals

The animals were 8 male Sprague-Dawley albino rats, weighing between 250–300 g at the time of surgery, housed in individual cages and maintained on ad lib water and food schedule.

Apparatus and Procedure

Transparent acrylic plastic boxes for operant conditioning in rats (Skinner box, Scientific Prototype) (size 23.60 x 20.32 x 19.5 cm) with a single lever were used. The system was programmed to deliver a brief 0.25 sec 60 Hz sine wave, 100 μA (peak-to-peak) (35.7 μA RMS) electrical pulse in the brain via indwelling bipolar electrodes with each lever press. Oscilloscope measurements of current were

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taken at the beginning of each training and experimental session in order to verify intensity. Twisting of leads was prevented by the use of a commutator-swivel system.

Subjects were anaesthetized with sodium pentobarbital (50 mg/kg, ip). Surgical procedures followed those described by Olds and Milner [21]. Bipolar stainless steel wires (0.254 cm in dia.) insulated except at the cross-section of their tips, were aimed stereotaxically to terminate the exposed tip in the posterior lateral hypothalamus (PLH) at coordinates 2 mm posterior to bregma, 1.4 mm lateral to the sagittal suture, and 9 mm down from the top of the skull. The incisor bar was level with the interaural plane. After a 10-day recovery period, animals were trained in Skinner boxes to self-stimulate for a period of 20 daily sessions of 30 min in length until free responding maintained a relatively constant rate for three consecutive days.

On the experimental day, animals were allowed to self-stimulate freely for a period of 30 min. Following this pretest period, the rats were injected either with saline (Vehicle (V)) or DL-alpha-methyl-para-tyrosine methyl ester (α-MPT) (Regis Chemical Co., Chicago) (100 mg/kg). Alpha-MPT and apomorphine (A) solutions were prepared by dissolving α-MPT or A in 0.9% NaCl. All drugs and vehicle were administered ip at room temperature. A period of 10 min was allowed for injections. Following the injection period, animals were permitted to continue SS for the next 30 min. They were then returned to their home cages for a period of 2 1/2 hr. Subsequently, the rats were returned to their Skinner boxes and another 30 min reading was taken. They were then injected, at time 4 hr, either with V or with A at dosages of 0.25 mg/kg (A 0.25) or 0.5 mg/kg (A0.5). Eight 30 min readings were then taken. The 30 min sessions for all animals commenced with five forced intracranial stimulations in order to verify current intensity which was set at 100 μA peak-to-peak for all animals. With the exception of the time alloted for injections and rest period, the animals spent the complete 7 hr 40 min experimental time in their Skinner boxes. As can be seen in Fig. 1, there were six combinations of drug treatments. Each of the 8 animals received all six treatments in randomized order. Between each treatment there was a one week recovery period during which the rats were allowed to self-stimulate; no significant variations of SS rates were observed in subjects during recovery period when compared to their pretreatment performance. The response outputs for each 30 min session of the same animal were used in calculating the different scores across treatments at a given time reading. The Wilcoxon Matched-pairs Signed-ranks test [12] was used for the purpose of statistical analysis.

Upon completion of the experiment, subjects were killed with an overdose of sodium pentobarbital solution (200 mg/kg). The brains were fixed in formaldehyde (10%); transverse sections were made and stained according to the luxol fast blue technique of Kluver Barrera [14] for histological verification of electrode positions.

RESULTS

The so-called PLH probes fell either in the posterior lateral hypothalamus at the level of the mamillary bodies or in the ventromedial tegmentum (VMT) in two cases both in or around the medial forebrain bundle, the substantia nigra and the area of Tsai.

The results of the statistical findings on the effects on SS

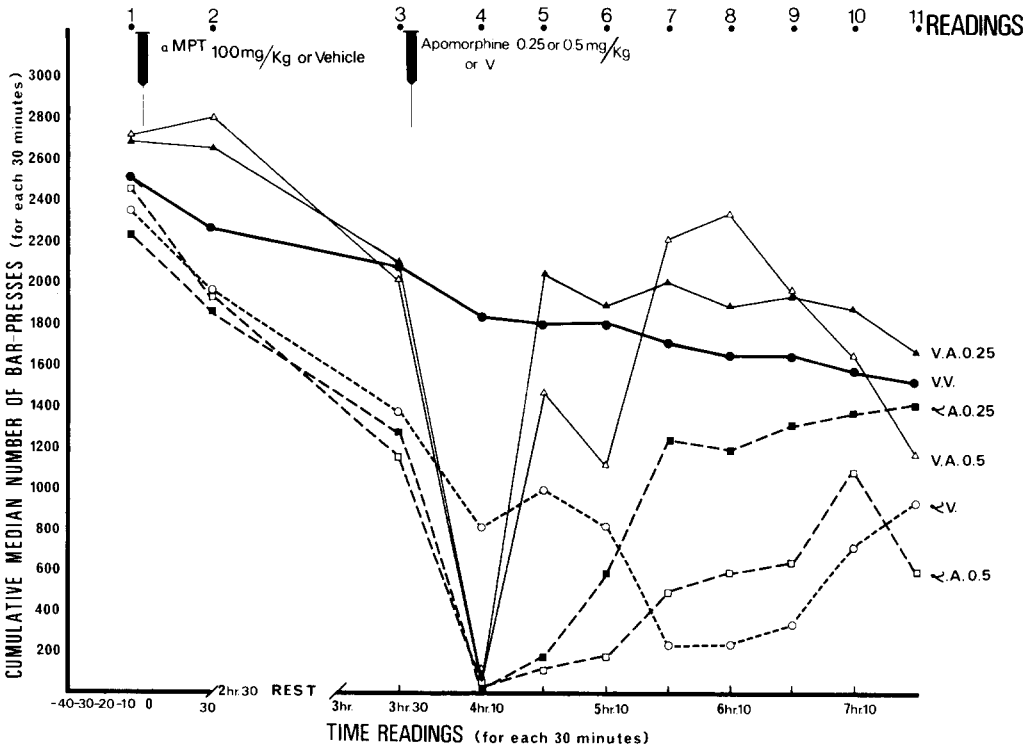

FIG. 1. Summary of the effects of α-MPT and Apomorphine (A) on SS rates. Alpha-MPT, 100mg/kg or saline vehicle (V) were injected ip at time − 0:10 hr after a control reading of 30 min. Apomophine, 0.25 mg or 0.5 mg/kg, or V were administered at 3:40 hr. Subsequent readings were taken every 30 min.

are summarized in Fig.1. As the effects of A on VMT animals did not differ from those on PLH animals their results were grouped together. Significant differences were observed at every 30 min reading from 0:30 hr following the first injection through to 7:10 hr (Readings 2 through 10), while no significant differences were found at the reading 10 min before the first injection or at the last reading, 7:40 hr (Readings 1 and 11) for any group. The summary of comparisons of paired treatments using the Wilcoxon test [12] is illustrated in Table 1.

Effect of α-MPT on SS

At the time 0:30 hr following one injection of α-MPT and again at the reading at 3:30 hr (Readings 2 and 3), there was a significant drop in bar-pressing rates. At 4:10 hr (30 min following the second injection), a significant difference was noted between the V-V treatments (control) and the α-MPT-V treatments; in fact, significant differences were observed between these two sets of treatments from 0:30 hr through 7:10 hr, inclusive (Readings 2 through 10).

Effect of Apomorphine on SS and Reversal of α-MPT Effect

Regardless of the pretreatment (α-MPT or V), a single injection of A 0.25 or A 0.5 caused an immediate suppression of SS behavior which lasted from 4:10 hr to 4:40 hr for the V-A 0.25 group and from 4:10 hr to 5:10 hr for the V-A 0.5 and α-A groups (Readings 4 and 5, and Readings 4, 5 and 6, respectively). At 4:40 hr, rats which were pretreated with V and received injections of A0.25 or A 0.5 made significantly more bar presses than animals which were pretreated with α-MPT and received A 0.25 or A 0.5 (Reading 5). Also at 4:40 hr, significant differences were observed between the V-V group and all other groups except V-A 0.25. Finally at 4:40 hr, V-A 0.25 animals were found to make significantly more bar presses than V-A 0.5 animals and α-MPT-V animals.

At 5:10 hr, the V-V and V-A 0.25 rats made significantly more bar presses than either the α-MPT-A 0.25 or α-MPT-A 0.5 aminals (Reading 6). At this same time, V-A 0.25 and V-A 0.5 animals made significantly more bar presses than α-MPT-A 0.5 animals made but not significantly more than α-MPT-A 0.25 animals. Finally, V-A 0.25 rats made significantly more bar presses than V-A 0.5 animals at 5:10 hr.

At 5:40 hr and 6:10 hr, α-MPT-A 0.25 rats made significantly more bar presses than α-MPT-V animals. Also at 5:40 hr and 6:10 hr, V-A 0.25 and V-A 0.5 rats made significantly more bar presses than either α-MPT-A 0.5 or α-MPT-A 0.25 animals.

At 6:40 hr animals receiving V-A 0.25 or V-A 0.5 or α-MPT-A 0.25 or α-MPT-A 0.5 made significantly more bar presses than animals receiving α-MPT-V. At 7:10 hr, rats receiving V-A 0.25 or V-A 0.5 or α-MPT-A 0.25 made significantly more bar presses than animals receiving α-MPT-V.

Concerning spontaneous behavior, within 2 to 3 min after injection of A it was noted that the rats showed an increase of spontaneous exploratory behavior (with sniffing, rearing, walking or standing) when compared with V or α-MPT animals.

TABLE 1

LEVELS OF SIGNIFICANCE IN THE WILCOXON MATCHED–PAIRS SIGNED–RANKS TEST (ONE–WAY TEST)

	1	2	3	4	5	6	7	8	9	10	11
TIME READINGS	–0:10 hr	0:30 hr	3:30 hr	4:10 hr	4:40 hr	5:10 hr	5:40 hr	6:10 hr	6:40 hr	7:10 hr	7:40 hr
Paired Treatments											
VV – α V	–	0.025	0.005	0.005	0.025	0.01	0.005	0.005	0.025	0.025	–
VV – α A 0.25	–	0.005	0.005	0.005	0.005	0.005	–	–	–	–	–
VV – α A 0.5	–	0.005	0.005	0.005	0.005	0.005	–	–	–	–	–
VV – VA 0.25	–	–	–	0.005	–	–	–	–	–	–	–
VV – VA 0.5	–	–	–	0.005	0.01	–	–	–	–	–	–
α V – α A 0.25	–	–	–	0.005	0.025	–	0.025	0.01	0.01	0.01	–
α V – α A 0.5	–	–	–	0.005	–	–	–	–	0.01	–	–
α V – VA 0.25	–	0.005	0.025	0.005	–	–	0.01	0.005	0.005	0.025	–
α V – VA 0.5	–	0.005	0.025	0.005	–	–	0.025	0.025	0.005	0.025	–
α A 0.25 – α A 0.5	–	–	–	–	–	–	–	–	–	–	–
α A 0.25 – VA 0.25	–	0.005	0.005	–	0.005	0.01	–	–	–	–	–
α A 0.25 – VA 0.5	–	0.005	0.005	–	0.01	–	–	–	–	–	–
α A 0.5 – VA 0.25	–	0.005	0.005	–	0.005	0.005	0.005	0.01	–	–	–
α A 0.5 – VA 0.5	–	0.005	0.005	–	0.01	0.025	0.025	0.025	–	–	–
VA 0.25 – VA 0.5	–	–	–	–	0.01	0.005	–	–	–	–	–

DISSCUSSION

Our results indicate that apomorphine can reverse the suppressive effects of α-MPT on SS; this was particularly evident with the lower dose of apomorphine (0.25 mg/kg). However, after injection of apomorphine subsequent to saline administration, animals showed a transitory suppression of SS which was longer lasting with the higher dose of apomorphine (0.5 mg/kg). The animals were then observed to show very intense exploratory activity commencing 2–3 min after injection. The transitory depressive effects of apomorphine on SS that we observed concur with the report of Butcher and Anden [5] who found that apomorphine caused a depressive effect on bar pressing with a variable-interval schedule of reinforcement. Following injection of apomorphine subsequent to α-MPT administration, SS was further depressed, followed by an increase of bar-pressing rates. The later occuring stimulating effects of apomorphine on SS that we noted are in agreement with the observation of Butcher [4] who reported increased bar pressing in a free-operant avoidance situation. The depressive effects of apomorphine were thought to be due to intrusion of such phenomena as exaggerated sniffing [5], while the stimulating action was thought to be related to facilitation of goal-directed behavior [11]. However, as Carlsson has suggested [6], the view that suppressive effect of apomorphine might be due to an induction of competing, stereotypical behaviors might be only partially correct. His data suggested that, while apomorphine induces enhanced sniffing and exploration, it also gives decreased habituation; hence, apomorphine at a high dose would give an increased and persistant reactivity to many types of stimuli making the necessary focus on the goal stimulus less probable. On the other hand, at a low dose the increased exploration would facilitate SS. It is somewhat surprising that, although the effect on exploration is almost immediate, the reinstatement of SS after α-MPT injection became significant only after 2 hr. Possibly a lower dose of apomorphine, such as 0.125 mg/kg would reinstate SS more quickly.

The mechanisms of action of apomorphine on SS are possibly dopaminergic as the suppression of SS induced by α-MPT, which decreases brain catecholamine levels does not give a complete blockade at the dopamine receptors; hence, their direct stimulation by apomorphine would reinstate SS. Although our experiment supports the hypothesis of Van Rossum and Hurkmans [32] according to which an increase in motility is a result of stimulation of dopamine receptors, an essential interaction with noradrenaline cannot be eliminated in the light of the works of many researchers [2, 9, 22, 23, 29] which suggest that the noradrenergic neurons are involved in the stimulation of motility.

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