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BRIEF COMMUNICATION

Serotonergic Basis of Reward in Median Raphé of the Rat[1]

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MILIARESSIS, E. *Serotonergic basis of reward in median raphé of the rat.* PHARMAC. BIOCHEM. BEHAV. 7(2) 177–180, 1977. – Rats were trained to self-stimulate simultaneously in the ventromedian tegmentum (VMT) and the median raphé (MR) by pressing two independent bars. Bar-pressing rates for VMT self-stimulation (SS) were increased following methamphetamine and decreased following α-methyl-para-tyrosine while no appreciable effects were observed on SS in the MR. On the other hand, MR SS was specifically decreased following para-chlorophenylalanine. The above results taken together support the hypothesis that SS in the MR is due to the stimulation of serotonergic neuronal elements.

Self-stimulation Median raphé p-Chlorophenylalanine Ventral tegmentum
Methamphetamine α-Methyl-p-tyrosine

PHARMACOLOGICAL attempts to affect self-stimulation behavior (SS) with serotonergic drugs have led to contradictory conclusions (for a brief review see Miliaressis *et al.* [6]. This markedly contrasts with the relatively uniform results obtained with drugs known to act on catecholaminergic mechanisms. In addition, SS in the rat is often elicited with electrodes located in brain regions containing mainly catecholaminergic neuronal elements. The consistency between neuroanatomical and biochemical data led progressively to a catecholaminergic hypothesis of SS [3]. However, we reported recently that electrodes in the median raphé (MR) produced very high rates of SS and that this behavior was inhibited following blockade of serotonin synthesis by para-chlorophenylalanine [6]. In an additional recent work we further reported that SS in the MR was accompanied by dramatic hyperthermia [7]. In view of the role of serotonin in thermoregulation [9], this last observation provides additional support that SS in the MR is elicited by serotonergic stimulation. In the present work we report pharmacological data of SS obtained in rats which were allowed to self-stimulate simultaneously in serotonergic and catecholaminegic brain sites. Simultaneous SS via two electrodes represents a sensitive and accurate technique in the evaluation of the relative reward value of the stimulation in different brain sites [5]. This technique may be very useful in SS experiments whereby drugs are expected to produce differential effects on distinct brain areas. An inherent advantage of this method is that non-specific drug effects as well as intertrial variations due to sickness or other undesirable reasons can be easily detected. In view of these advantages, this technique was used in the prsent pharmacological work in an attempt to compare the neurochemical correlates of SS elicited in a classical catecholaminergic area (VMT) and in a serotonergic region where SS was recently reported [6].

METHOD

The experiments were performed on 4 male, Sprague-Dawley rats (300 g), implanted with two bipolar electrodes (200 μM in diameter) in the median raphé (MR), and the ventromedian tegmentum of the mesencephalon (VMT), respectively. With the incisor bar 3 mm above the interaural line, stereotaxic coordinates for the VMT were 4.3 mm posterior to the bregma, 0.4 mm lateral to the midline, and 8.4 mm under the surface of the skull. Median raphé coordinates were respectively, 6.0 mm, 0.0 mm and 8.0 mm. A week after surgery, rats were trained to self-stimulate in the VMT and MR regions by pressing two 3 × 5 cm independent levers in a 25 × 25 cm box. Both levers were at the same side of the box and 12 cm spaced. Depression of each lever delivered a 60 Hz sin. current of 250 msec duration. The intensity of the stimulation was adjusted in order to elicit similar but low barpressing rates

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in VMT and MR regions. (MR: 78.2 ± 21.9 μa; VMT: 91.2 ± 48.0 μa.) After stabilization of SS of low rates, rats were allowed to self-stimulate for a 110 min period for two consecutive days. Thirty minutes after the beginning of each SS period, rats received an IP injection of either normal saline (first day), or 0.75 mg/kg of methamphetamine (second day). Three days later, VMT and MR current intensities were increased in order to produce high barpressing rats (MR: 111.7 ± 31.3 μa; VMT: 118.5 ± 58.0 μa.) After stabilization, rats were allowed to self-stimulate for a 180 min period for two consecutive days. Thirty min after the beginning of each SS period, rats received an IP injection of either normal saline (first day) or 200 mg/kg of α-methyl-para-tyrosine (α-MPT, second day). Following five days of retraining (180 min SS periods), rats received an IP injection of 400 mg/kg of para-chlorophenylalanine-methyl-esther (pCPA). Barpressing rates obtained 24 hr following the injection were then compared to those obtained on the last control day. At the end of the experiments, the rats were sacrificed and the brains were sliced and stained with thionine for the purpose of macroscopic determination of electrode placements.

RESULTS

Figure 1 shows that VMT electrodes were implanted around the interpeduncular nucleus in a region that corresponds to the A10 dopaminergic cell group of Dahlström and Fuxe [1]. Median raphé electrodes were located in the upper part of the nucleus centralis superior (B8 serotonergic nucleus of Dahlström and Fuxe [1]).

Figure 2 shows the mean comparative effects of methamphetamine, α-MPT and pCPA on VMT and MR SS. A low dose of methamphetamine (Fig. 2a) resulted in an immediate dramatic increase of VMT SS while SS in the MR showed a slight compensatory decrease. Following α-MPT treatment (Fig. 2b), MR SS was essentially unaffected while barpressing rates for VMT SS were progressively inhibited. Figure 2c shows that barpressing rates for VMT and MR SS were identical prior to drug treatment. However, 24 hr following pCPA, MR SS was partially inhibited while barpressing rates for VMT SS showed a slight concomitant increase. All rats showed all of the effects described above except in the case of methamphetamine whereby one of the rats showed, in addition, a partial increase in MR SS.

DISCUSSION

In a previous work, we reported that rats with electrodes in the upper part of the MR may reach extremely high rates of SS [6] (above 6,000 presses/30 min). This finding that was recently confirmed by other investigators [10] underline this brain area as potentially significant in reward phenomenon.

The fact that rats perform simultaneous SS in the VMT and MR regions represents an intersting observation since this rules out any acute reciprocal inhibition between the rewarding activities of these two brain areas.

As shown in Fig. 1a, a low dose of methamphetamine results in a dramatic but specific increase of SS in the VMT area. In a recent experiment, we have reported that higher doses of this drug produce facilitation of MR SS as well [6]. This apparent discrepancy may mean however that high doses of methamphetamine release also serotonin at the terminals. An additional explanation could be that SS in the MR is initiated by stimulation of serotonergic neurons whose activity is partially relayed by catechola-

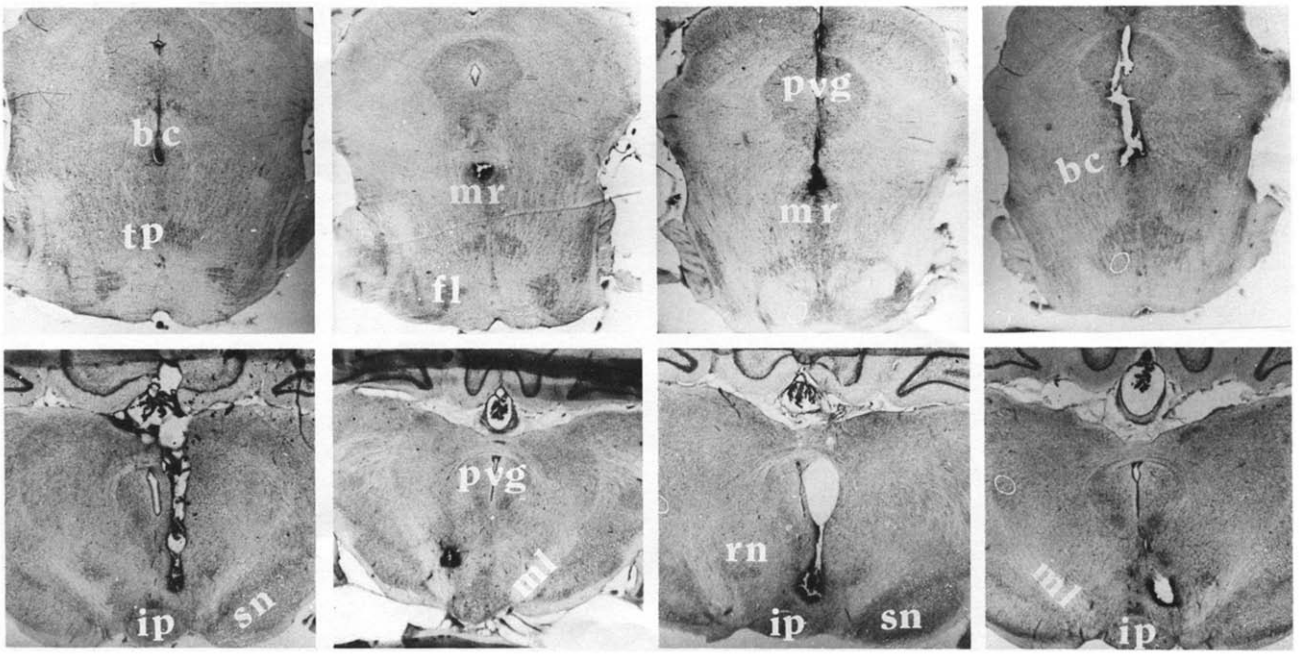

FIG. 1. Microphotographs showing the location of the electrodes in the median raphé and ventro-medial tegmentum. bc: branchium conjectivum; fl: fasciculus longitudinalis; ip: nucleus interpeduncularis; ml: lemniscus medialis; rn: nucleus ruber; pvg: substantia grisea centralis; su: substantia nigra.

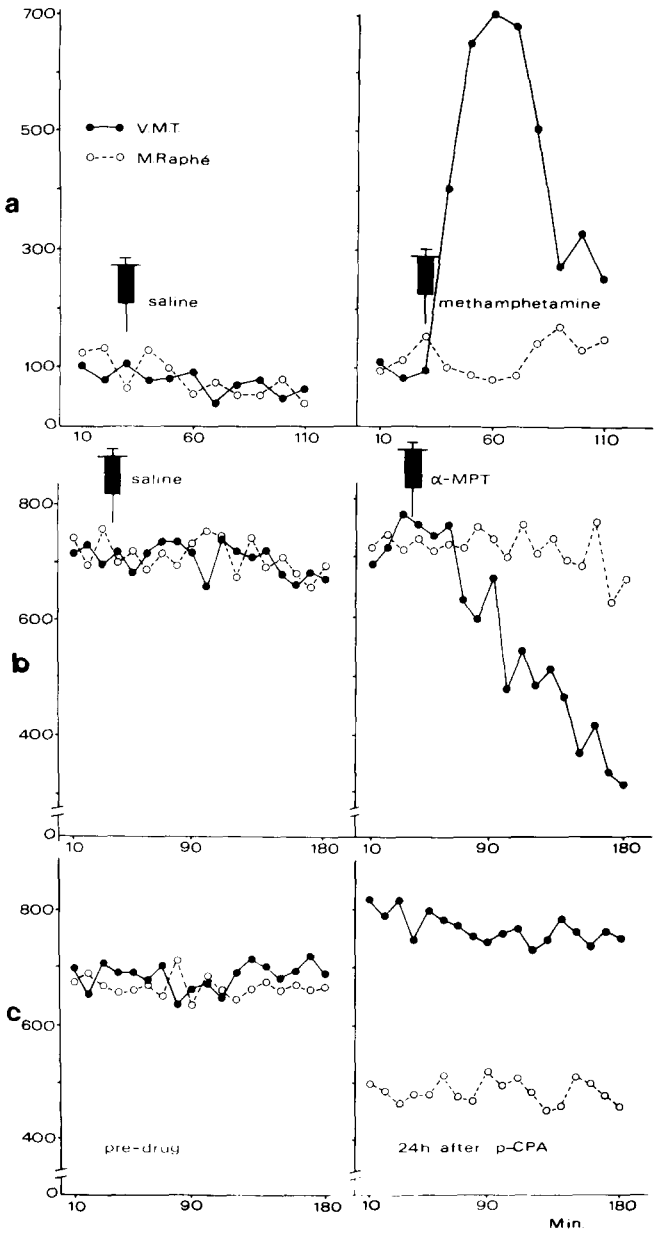

FIG. 2. Comparative effects of (a) methamphetamine, (b) α-methyl-paratyrosine, and (c) parachlorophenylalanine on bar-pressing rates for intracranial self-stimulation in the ventromedian tegmentum (VMT) and median raphé (MR) of the rat. The animals were implanted with two independent electrodes and they were allowed to perform simultaneous self-stimulation by pressing two separate levers respectively connected to VMT and MR electrodes. Ordinate: mean bar presses for 4 rats. For further details, see text.

minergic neuronal elements. This hypothesis cannot be currently excluded since such an anatomical arrangement has not been ruled out. The idea that SS may be mediated by more than one amine might be of interest since in pharmacological studies this behavior is implicitly supposed to involve a single neurotransmitter. The present results are in agreement with the data of Liebman and Butcher who showed that low doses of amphetamine produce dramatic increase of SS in the MFB but have no effect on SS elicited in the dorsal raphé [4].

As shown in Fig. 1b, α-MPT inhibits SS in the VMT but not in the MR. The rapid onset of the inhibitory effect, as shown in the present work, is in agreement with other SS data obtained in the VMT area [12]. The gradual extinction of VMT SS probably reflects the time course of catecholamine-synthesis blockade. A late inhibitory effect of α-MPT on MR SS cannot be excluded in the light of the present results. Such an effect however would be difficult to interpret, in view of the well-known severe motor impairment produced by this drug a few hours following injection.

The specific inhibitory effect of pCPA on MR SS as shown in Fig. 1c confirms our previous report [6]. However, in this last work, a gradual inhibition was observed. This discrepancy probably results from the difference in experimental schedules. A possible additional reason is that pCPA has also a slight facilitatory effect on VMT SS.

The pharmacological data reported in the present work are therefore consistent with the idea that SS in the MR is produced by activation of serotonergic neurons. However, Simon *et al.* have recently reported that SS in the dorsal raphé (DR) is increased by pCPA and decreased by α-MPT [10]. Six out of 12 electrodes in this last study were located in the very inferior part of the DR which is less than 1 mm above the location of our electrodes (in the upper part of the MR). Though numerous methodological differences between these two experiments could partially explain a few points of the contradictory results, it seems more probable that the fine localization of the electrodes is the decisive factor, especially in the raphé which represents an heterogeneous area.

In conclusion, the use of a sensitive behavioral technique in combination with different drugs enable us to demonstrate that positive reward can be obtained by stimulation of catecholaminergic (VMT) as well as serotonergic (MR) areas. In addition, the fact that SS of neither structure depended upon intact neurotransmitter mechanisms of the other suggest that these two reward systems are relatively independent.

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