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Circling Behaviour Induced by Electrical Stimulation of the Medial Forebrain Bundle, Importance of Stimulus Parameters and Dopaminergic Processes

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VAN DER HEYDEN, J. A. M. *Circling behaviour induced by electrical stimulation of the medial forebrain bundle, importance of stimulus parameters and dopaminergic processes.* PHARMACOL BIOCHEM BEHAV 21(4) 567–574, 1984.—Unilateral electrical stimulation of the substantia nigra or the ventral tegmental area produced postural asymmetry and increased locomotor activity respectively. Concurrent stimulation of the efferent pathways of these two areas (medial forebrain bundle) resulted in contraversive circling behaviour that was dependent upon the current intensity and frequency of the stimuli. The application of biphasic electrical pulses minimised the damage to the brain site stimulated; no decrease in circling intensity over time, nor spontaneous circling after administration of amphetamine or apomorphine without stimulation was observed. The contraversive circling behaviour was induced by activation of the ascending dopaminergic pathways as revealed by the close correlation between the site of stimulation and the localisation of this pathway, its antagonism by haloperidol and its abolishment by pretreatment with reserpine and α-methyl-p-tyrosine. Apomorphine likewise inhibited the electrical stimulation-induced circling behaviour. These results are discussed with regard to the influence of the stimulation parameters and the dopaminergic processes involved.

Electrical stimulation Medial forebrain bundle Contraversive circling Stimulus parameters Apomorphine Haloperidol

UNILATERAL electrical stimulation of the ascending dopaminergic projection causes contraversive circling behaviour or body asymmetry [2, 3, 4, 5, 25]. This electrical stimulation-induced behaviour is associated with an increase of the striatal dopamine release [2, 18, 20, 27] and can thus be blocked by neuroleptic drugs [4, 25, 29]. In general, turning behaviour in the rat cannot be elicited by stimulation of the A10 mesolimbic dopamine pathway alone [2, 9, 11, 14]. Two functional dopamine components are required: a striatal dopamine imbalance, causing a postural asymmetry, and stimulation of the mesolimbic dopamine systems, in particular the nucleus accumbens, which provides a locomotor component and thus regulates the rate of rotation [15,24]. By implanting a bipolar electrode in the ascending dopamine pathway, rostral to the substantia nigra, it is possible to stimulate simultaneously these two systems and thus to induce turning behaviour. Though the effects of agents that manipulate central dopamine systems can be studied in many other models of circling behaviour, this procedure requires no development of receptor supersensitivity or the administration of agents to induce circling. Furthermore, since the neurones are being stimulated directly, receptor-mediated feedback inhibitory systems are circumvented. In the present paper the effect of the stimulus parameters and the site of stimulation, as well as the effect of both pre- and postsynaptic dopamine agonist apomorphine and the dopamine antagonist haloperidol, were investigated.

METHOD

Animals

Male Wistar rats (TNO, Zeist, The Netherlands) were used. The animals were individually housed. At the time of surgery, the animals weighed between 175 and 225 grams.

Electrodes

The electrodes were made by twisting trimel coated stainless steel wire (diameter 0.2 mm) tightly together. The electrodes were insulated over the entire length except for the cross section at the tip. The electrodes were soldered to small plugs, mounted in a teflon holder and were checked for leakage before implantation.

Surgical Procedures

The rats were anaesthetized with chloral hydrate (300 mg/kg IP) and mounted in a stereotaxic instrument (De Koningh, Arnhem, The Netherlands) according to König and Klippel [17]. A burr hole was made in the exposed skull to allow the introduction of stimulation electrodes. The coordi-

nates (according to König and Klippel, [17]) of the brain areas under investigation were: substantia nigra A 2.2 mm, L 2.0 mm, V −2.1 mm; ventral tegmental (A10) area A 2.2 mm, L 0.6 mm, V −2.5 mm; medial forebrain bundle (MFB) A 4.0 mm, L 1.6 mm, V−2.7 mm. The electrode with connector was fixed on the skull with small jeweler's screws and acrylic dental cement.

Electrical Stimulation

Biphasic square-wave pulses were delivered by two Grass PSIU6 constant-current sources connected to a Grass S88 stimulator. The electrodes were connected to the stimulator by a mercury swivel. The current delivered was continuously monitored by an oscilloscope connected across a 1 K-ohm resistor in series with the electrode. Duration and current intensity of both phases were always carefully equalized to minimize net charge flow and electrode polarization. The standard stimulation parameters used were: pulse duration 0.4 msec, pulse interval duration 0.2 msec. In experiments, where the current intensity (presented as amplitude of one phase, 40–400 μA), pulse duration (0.05–1 msec) or pulse interval duration (0–10 msec) was varied, the frequency was held constant at 40 Hz. In experiments, where the frequency was varied (between 5 and 100 Hz), the current intensity was 40 μA above the threshold value, that is defined as the current intensity, at a frequency of 40 Hz at which the animals circled at a rate of $\geq$4 turns/min. This threshold value is different, but constant for each animal.

After approximately seven days for postoperative recovery, the rats were tested for a behavioural response to electrical stimulation. The animals were placed in a 25×40 cm perspex cage and stimulated for 30 sec, starting at a current intensity of 40 μA. The 30 sec stimulation was applied at 90 sec intervals; for each successive stimulation period the current intensity was increased by fixed steps of 40 μA to a maximum of 400 μA. In experiments where pulse- or pulse interval duration were varied, the same time schedule was used. Only those animals, with electrodes implanted in the MFB that exhibited consistent contraversive turning (maximum circling intensity $\geq$20 turn/min) were used. Animals were placed in a circular cage (25 cm diameter) and contraversive turning behaviour was recorded by direct observation with only complete rotations recorded. The criteria for animals with electrodes in the substantia nigra or ventral tegmental area were: head turning and increased locomotor activity respectively. The latter activity was measured by placing the perspex cage on a Animex motility meter (LKB). When testing drugs, the animals served as their own control by first stimulating to obtain a stimulus-response curve, followed by intraperitonal administration of saline or the drug. The stimulus-response curve was measured again 10 or 20 min later.

Histology

After completion of the experiments, all animals were lesioned through the implanted electrode with a 1 mA monophasic pulse of 10 sec. The animals were deeply anaesthetized with chloral hydrate and perfused intracardially with saline followed by 4% formalin. The brains were frozen and sectioned with a freezing microtome into serial 100 μm coronal slices after which the localisation of the electrode tip was examined.

TABLE 1

EFFECT OF UNILATERAL STIMULATION OF THE VENTRAL TEGMENTAL (A10) AREA ON LOCOMOTOR ACTIVITY IN THE RAT

Current Intensity (μA)	Locomotor Activity (counts/30 sec) Pre-Stimulation	Stimulation
40	9 ± 4	14 ± 4
80	15 ± 4	48 ± 3*
120	13 ± 4	55 ± 5*

Values are the mean ± SEM of 6 experiments. The frequency was 40 Hz.

*$p<0.05$ compared to pre-stimulation value.

Expression of Results

The locomotor activity for rats with electrodes in the ventral tegmental area is given as the mean number of counts during the stimulation period (30 sec) and the preceding 30 sec of non-stimulation period.

Two kinds of experiments were performed with rats that had electrodes implanted in the MFB: variation of the current intensity at a frequency of 40 Hz and variation of the frequency at 40 μA above the threshold current intensity. The circling intensity is calculated as a percentage of the maximal response for each animal. In the former kind of experiments the current intensity at which the rats showed $\geq$90% of the maximal effect that is reached when the circling intensity increased less than 10% in three consecutive steps is chosen as a reference point (step 5). The average stimulus-response curves (see Fig. 2), were then calculated by superimposing the individual stimulus-response curves. In the latter kind of experiments, the current intensity chosen (40 μA above threshold) is such, that approximately the same number of rotations (16 turns/min) is induced at a frequency of 40 Hz. For each experiment the average maximum response and the corresponding stimulus parameter at >90% of this maximum is given.

In the experiments where drugs were administered, each animal served as its own control and the mean change in circling intensity (difference between circling intensity after and before administration of the drug) is presented. The Wilcoxon matched pair signed rank test was used for statistical analysis. In combined drug treatment experiments the Mann-Whitney U test was used.

RESULTS

Electrical Stimulation of the Ventral Tegmental Area of Substantia Nigra

Unilateral stimulation of the ventral tegmental (A10) area produced an increased locomotor activity without postural asymmetry or head turning. The minimal current intensity required for this effect was 80 μA, at the stimulus parameters: frequency 40 Hz, pulse duration 0.4 msec, pulse interval duration 0.2 msec. In Table 1, the mean locomotor activity preceding stimulation and during stimulation is presented.

Unilateral stimulation of the substantia nigra (zona compacta) resulted in contraversive head turning and postural asymmetry at a current intensity of 100±11 μA (mean±SEM of 6 experiments). The other stimulus parameters were similar to those used in the above described experiment. Stimu-

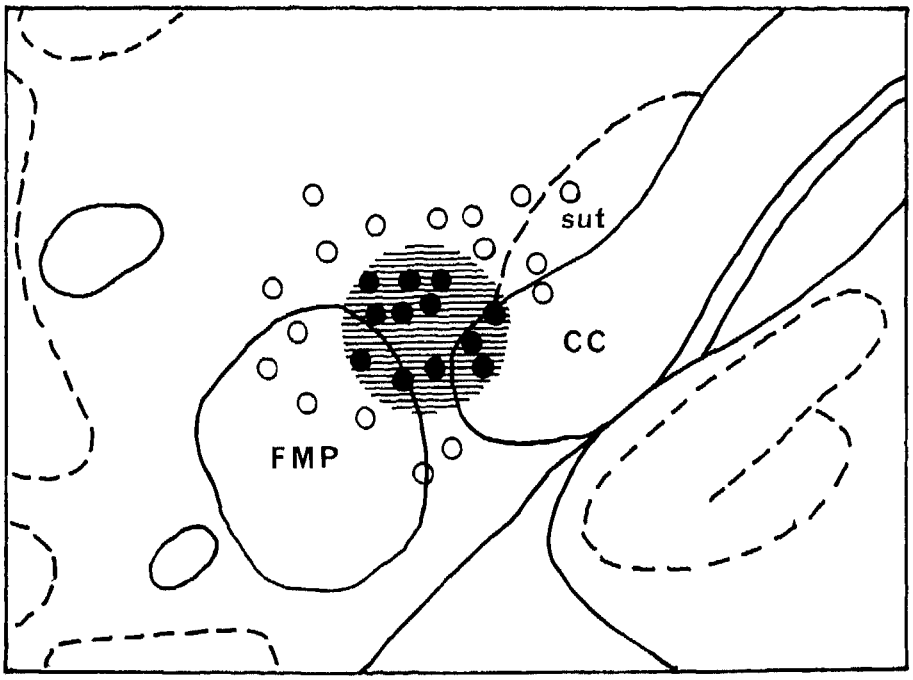

FIG. 1. Localization of electrodetips aimed at the MFB. Filled circles indicate placement of the electrode tips, causing the animal to turn contraversive upon stimulation, open circles indicate not responding animals. Section is redrawn from König and Klippel [17] at A 3750 μm. Abbreviations: CC, crus cerebri; FMP, fasciculus medialis prosencephali; sut, nucleus subthalamicus. The shaded area indicates localization of the ascending dopaminergic axons (MFB) according to Ungerstedt [26].

lation at higher current intensities produced longitudinal rolling, but no or little (<4 turns/min) contraversive circling behaviour.

Histological examination revealed that all electrode tips were localized in the desired region.

Electrical Stimulation of MFB

Site of stimulation and variation of stimulus parameters. Unilateral electrical stimulation of the MFB produced contraversive circling behaviour. The localization of the electrode tips of animals that had electrodes implanted aimed for the MFB, is presented in Fig. 1. Also shown in this figure is the localization of the ascending dopaminergic axons at this section of the brain [26]. An electrode placement was considered successful if the animal showed consistent contraversive circling (≥20 turns/min) at a current intensity not higher than 320 μA. Animals that did not reach this intensity of contraversive circling often showed a postural asymmetry at higher current intensities without circling. The threshold current intensity at a frequency of 40 Hz that produced contraversive circling under our experimental conditions was 105±7 μA (mean±SEM of 41 experiments). This threshold value is strongly dependent upon the stimulus parameters used. In Fig. 2 the influence of both pulse duration and pulse interval duration is presented. Increasing the pulse duration at a fixed pulse interval duration of 0.2 msec leads to an enhanced circling response. Only at a relatively large pulse interval duration (3.2 msec) at a fixed pulse duration of 0.4 msec an increase in circling intensity was observed.

Both increasing the current intensity and frequency produced S-shaped stimulus effect-curves (Fig. 3) i.e., as

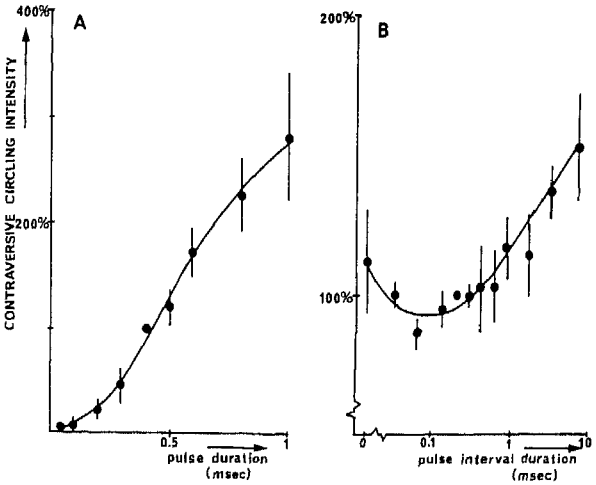

FIG. 2. Effect of pulse- and pulse interval duration on contraversive circling behaviour. The presented values are the means±SEM of 6 experiments. A. Influence of pulse duration at a fixed pulse interval duration of 0.2 msec on contraversive circling induced by electrical stimulation of the MFB. The effect at a pulse duration of 0.4 msec is set at 100% the current intensity was 130±22 μA. B. Influence of pulse interval duration at a fixed pulse duration of 0.4 msec on contraversive circling behaviour. The effect at a pulse interval duration of 0.2 msec is set at 100%, the current intensity was 170±3 μA.

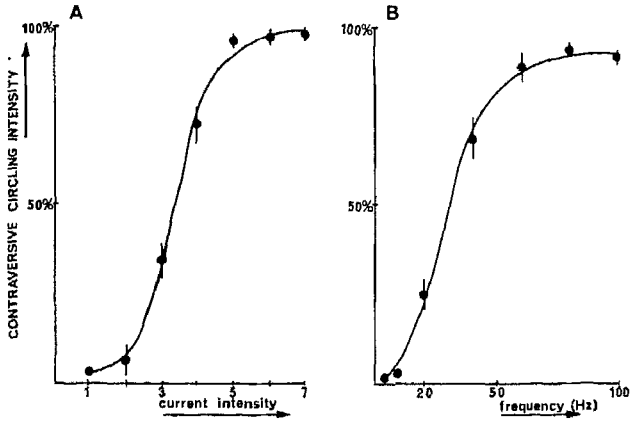

FIG. 3. Effect of variation of current intensity or frequency on contraversive circling behaviour. A. Influence of current intensity on contraversive circling induced by electrical stimulation of the MFB. The current intensity is increased in steps of 40 μA, the mean intensity (±SEM) of step 5 was 196±23 μA (12 experiments). The maximum circling intensity was 32±2 turns/min, the frequency was 40 Hz. B. Influence of frequency on contraversive circling induced by electrical stimulation of the MFB. The frequencies applied were: 5, 10, 20, 40, 60, 80 and 100 Hz at 40 μA above the threshold current intensity. The maximum response (28±3 turns/min) was reached at 61±3 Hz (mean±SEM of 12 experiments).

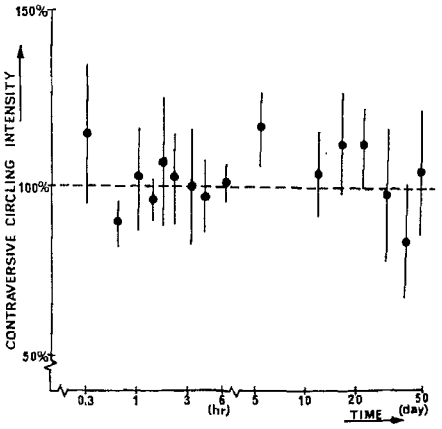

FIG. 4. Contraversive circling intensity after repeated stimulation. The circling intensity on $t=0$ (19±2 turns/min at 82±11 μA) is set at 100%. Time refers to the time after the first stimulation ($t=0$). The data shown are the mean±SEM of 6 animals. The stimulation frequency was 40 Hz.

TABLE 2

CONTRAVERSIVE CIRCLING DURING ELECTRICAL STIMULATION OF THE MFB: EFFECT OF APOMORPHINE AND HALOPERIDOL AT DIFFERENT CURRENT INTENSITIES

Treatment	Maximal Effect at Step 5	Current Intensity (steps of 40 μA) 1	2	3	4	5	6	7
Apomorphine 0.03 mg/kg	29 ± 3 (267 ± 17)	5 ± 6	6 ± 9	−3 ± 10	4 ± 10	−2 ± 6	2 ± 6	2 ± 6
Apomorphine 0.1 mg/kg	31 ± 4 (313 ± 7)	−2 ± 8	−4 ± 7	4 ± 7	11 ± 8	10 ± 4	7 ± 4	3 ± 5
Apomorphine 0.3 mg/kg	28 ± 3 (267 ± 17)	−3 ± 4	−13 ± 7	−11 ± 7	−14 ± 9	−22 ± 9*	−16 ± 4*	−12 ± 7
Apomorphine 1.0 mg/kg	41 ± 3 (167 ± 16)	−1 ± 1	−7 ± 8	−12 ± 3*	−38 ± 8*	−48 ± 12*	−36 ± 13*	−23 ± 13
Apomorphine 3.0 mg/kg	36 ± 5 (227 ± 41)	−11 ± 6	−16 ± 7	−28 ± 10*	−58 ± 8*	−83 ± 7*	−78 ± 3*	−72 ± 3*
Haloperidol 0.05 mg/kg	28 ± 3 (227 ± 20)	2 ± 1	−1 ± 3	−25 ± 15	−9 ± 10	−13 ± 15	−10 ± 11	−5 ± 9
Haloperidol 0.1 mg/kg	33 ± 5 (213 ± 27)	−2 ± 3	2 ± 2	3 ± 4	−3 ± 6	−6 ± 6	−6 ± 5	−2 ± 3
Haloperidol 0.2 mg/kg	36 ± 4 (220 ± 31)	7 ± 7	2 ± 3	−3 ± 2	2 ± 2	−6 ± 3	4 ± 1	2 ± 2
Haloperidol 0.4 mg/kg	35 ± 7 (220 ± 20)	−2 ± 2	−8 ± 4	−21 ± 6*	−52 ± 14*	−89 ± 10*	−86 ± 13*	88 ± 11*

The presented values (mean ± SEM of 6 experiments) are the differences between contraversive circling intensity after and before administration of the drug. Apomorphine and haloperidol were administered IP 10 min and 20 min respectively before the second stimulation session. Maximal effect refers to the maximal number of turns/min at the indicated current intensity (=step 5, between brackets). The frequency was 40 Hz.

*Indicates: $p<0.05$ (Wilcoxon).

TABLE 3

CONTRAVERSIVE CIRCLNG DURING ELECTRICAL STIMULATION OF THE MFB: EFFECT OF APOMORPHINE AND HALOPERIDOL AT DIFFERENT FREQUENCIES

Treatment	n	Maximal Effect	Frequency (Hz) 5	10	20	40	60	80	100
Apomorphine 0.03 mg/kg	6	24 ± 5 (63 ± 3)	0	7 ± 5	9 ± 9	8 ± 8	−3 ± 3	3 ± 5	6 ± 3
Apomorphine 0.06 mg/kg	6	23 ± 2 (57 ± 6)	0	−8 ± 3*	−19 ± 7*	−38 ± 9*	−13 ± 8	−15 ± 6*	−5 ± 5
Apomorphine 0.1 mg/kg	9	25 ± 3 (60 ± 3)	0	−2 ± 1	−15 ± 2*	−39 ± 6*	−47 ± 9*	−31 ± 7*	−31 ± 8*
Apomorphine 0.2 mg/kg	12	29 ± 2 (60 ± 6)	−1 ±1	−1 ± 1	−1 ± 5	−7 ± 9	−9 ± 7	−6 ± 6	−12 ± 5*
Apomorphine 0.3 mg/kg	6	26 ± 3 (63 ± 8)	0	1 ± 3	−9 ± 2*	−23 ± 5*	−9 ± 10	−3 ± 5	−6 ± 3
Apomorphine 1.0 mg/kg	6	26 ± 4 (57 ± 10)	0	−1 ± 1	−14 ± 9	−31 ± 15*	−12 ± 11	−6 ± 6	−3 ± 9
Apomorphine 3.0 mg/kg	6	25 ± 3 (60 ± 5)	0	−3 ± 2	−23 ± 10*	−31 ± 4*	−23 ± 7*	−25 ± 6*	−27 ± 9*
Haloperidol 0.05 mg/kg	6	26 ± 5 (63 ± 3)	1 ± 1	2 ± 3	−5 ± 3	−12 ± 11	−10 ± 7	1 ± 10	14 ± 11
Haloperidol 0.1 mg/kg	6	22 ± 5 (60 ± 5)	3 ± 2	−3 ± 3	−8 ± 3	−13 ± 12	−4 ± 11	−16 ± 8*	−13 ± 8
Haloperidol 0.2 mg/kg	6	24 ± 4 (63 ± 8)	0	−5 ± 3	−16 ± 5*	−15 ± 7	−20 ± 4*	−35 ± 5*	−44 ± 4*
Resperine and α MT	7	20 ± 2 (57 ± 5)	−1 ± 1	−8 ± 3	−32 ± 10*	−39 ± 11*	−78 ± 10*	−69 ± 8*	−70 ± 7*

The presented values (mean ± SEM) are the differences between contraversive circling intensity after and before administration of the drug. Apomorphine and haloperidol were administered IP 10 min and 20 respectively before the second stimulation session.

Reserpine (5 mg/kg IP) and α-methyl-p-tyrosine methylester were administered 34 hr and 3 hr respectively before the second stimulation session. n refers to the number of experiments, maximal effect refers to the maximal number of turns/min at the indicated frequency. The current intensity was 40 μA above threshold value. *Indicates: $p<0.05$ (Wilcoxon).

intensity or frequency increased, circling behaviour increased until an asymptote was reached. Repeated stimulation of the MFB did not result in damage to the tissue. This was shown by the fact that the contraversive circling intensity during electrical stimulation, using stimulus parameters that were held constant for each animal did not change up to 43 days after the first stimulation session (Fig. 4). Moreover, amphetamine (5 mg/kg IP) did not induce spontaneous ipsiversive circling after repeated electrical stimulation of the MFB.

Pretreatment of the animals with reserpine (5 mg/kg) together with α-methyl-p-tyrosine methylester (300 mg/kg) administered intraperitoneally 24 hr and 3 hr respectively before electrical stimulation almost completely abolished contraversive circling behaviour (Table 3).

Effect of apomorphine and haloperidol. The effect of apomorphine on electrical stimulation-induced circling, obtained by variation of the current intensity is shown in Table 2. As can be seen from these results, 0.3 mg/kg apomorphine slightly decreased the circling response at higher current intensities. At higher dosages also an attenuation of the electrical stimulation-induced circling at lower current intensities was obtained, resulting in a flattening of the stimulus-effect curve was obtained. Haloperidol did not affect the circling behaviour up to a dose of 0.2 mg/kg, 0.4 mg/kg produced a decreased response.

Different results were obtained after variation of the stimulation frequency (Table 3). A much lower dose of apomorphine (0.06 mg/kg) produced a clearcut decrease in circling intensity that was especially evident after the administration of 0.1 mg/kg (Table 3). Apomorphine in dosages between 0.2 mg/kg and 1.0 mg/kg proved only to be marginally effective, whereas the highest dose used (3.0 mg/kg) again produced a strong inhibition of the electrical stimulation-induced circling behaviour. Under these experimental conditions haloperidol inhibited the contraversive circling at a dose of 0.2 mg/kg, but not at lower dosages (Table 3).

The effect of haloperidol on apomorphine-induced inhibition of contraversive circling intensity is presented in Fig. 5. The dosages of haloperidol used did not affect the circling response themselves (compare Tables 2 and 3). The current intensity-dependent decrease in circling response after the administration of 1.0 mg/kg apomorphine was not influenced by haloperidol. The decrease observed after 3.0 mg/kg apomorphine was antagonized at higher current intensities (≥ step 5, statistically significant increase compared to apomorphine alone, $p<0.05$). The maximal circling intensities of animals treated with 1.0 mg/kg or 3.0 mg/kg apomorphine combined with 0.2 mg/kg haloperidol were 30±4 turns/min at 288±12 μA and 27±3 turns/min at 286±10 μA respectively. Both the decrease in frequency-dependent cir-

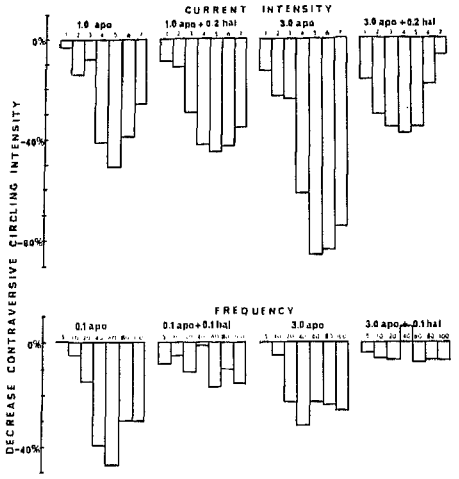

FIG. 5. Antagonism of apomorphine-induced effects by haloperidol during electrical stimulation of the MFB. The net decrease in contraversive circling intensity is presented: apo (dose in mg/kg IP indicated) refers to the decrease in circling intensity after apomorphine treatment minus the matching decrease after saline treatment. The results obtained after the combined treatment with apomorphine and haloperidol (apo+hal), administered 10 min and 20 min respectively before the second stimulation session are presented as the difference between the combined treatment and haloperidol treatment alone. In the top figure the results obtained after variation of the current intensity at 40 Hz are presented. In the bottom figure the results obtained after variation of the frequency at 40 μA above threshold current intensity are presented. All values are the means±SEM of 6 experiments.

cling intensity after administration of 0.1 mg/kg and 3.0 mg/kg apomorphine was antagonised by haloperidol (no significiant decrease compared to haloperidol alone). The maximal circling intensities of animals treated with 0.1 mg/kg or 0.3 mg/kg apomorphine combined with 0.1 mg/kg haloperidol were 27±4 turns/min at 70±4 Hz and 28±5 turns/min at 63±3 Hz respectively. Spontaneous circling behaviour after administration of drugs was never observed in the described experiments.

DISCUSSION

The results obtained during stimulation of the ventral tegmental (A10) area and/or the substantia nigra support the hypothetical model that an imbalance of striatal receptor activity leads to postural asymmetry that feeds into an amplifier system, the gain of which is related to receptor activity within the nucleus accumbens which accordingly regulates the rate of circling [15,24]. The increase in locomotor activity observed during stimulation of the ventral tegmental area can also be elicited by injecting dopamine into the nucleus accumbens [22,23]. Head-turning behaviour after electrical stimulation of the caudate nucleus, similar to that observed after stimulation of the substantia nigra described here is reported by Barnett and Goldstein [6]. Though several authors describe contraversive circling behaviour during stimulation of the substantia nigra [2, 4, 25], this might be due to concurrent stimulation of the nigrostriatal tract and the more medially located mesolimbic pathway.

We found a close correlation between the stimulation site producing contraversive circling behaviour and the location of the ascending dopaminergic fibers, as described by Ungerstedt [26]. We also found that pretreatment of the animals with reserpine and α-methyl-p-tyrosine, that strongly reduces the dopamine levels in the brain [27] almost completely abolished the electrical stimulation-induced circling behaviour. These findings, together with the observed antagonism by haloperidol confirm the involvement of the dopaminergic system in the electrical stimulation-induced contraversive circling behaviour.

The damage to the brain site that is electrically stimulated is minimized by applying biphasic pulses. Partial lesioning of the MFB would have resulted in spontaneous circling behaviour after administration of apomorphine or amphetamine and a decreased response to electrical stimulation [5,13]. Moreover, acute partial lesioning of the MFB will induce a dramatic increase in the release of dopamine [19,28]. The latter effect might explain the acute increase in circling response after repeated monophasic stimulation observed by Barghon and Costentin [5] and the fatigue of the turning response after 10 sec stimulation observed by Arbuthnott and Crow [2]. None of the above described effects have been observed under our experimental conditions.

Both the current intensity and the frequency of the electrical stimuli determined the contraversive circling intensity, resulting in S-shaped stimulus-effect curves. Though the physical inability of the animals to turn faster was an important factor in the maximal occurring circling intensity, this factor cannot completely explain the asymptotic results. Similar current intensity and frequency dependent stimulus-effect curves as those shown in Fig. 3, with a maximum asymptotic response of 50% could be obtained by respectively lowering the frequency by 20 Hz and the current intensity by 40 μA (data not shown). There is however, an important difference between the current intensity- and frequency-dependent circling rate. Increasing the current intensity beyond a threshold value will result in the activation of a larger population of axons, whereas an increase in the frequency (within the range used here) will lead to a more intense activation of the same axon population. Prolongation of the pulse duration also resulted in an increased circling response. This effect might be the result of either the increased amount of current passing through the electrode or a frequency-dependent-like effect. An increased response is also observed after relatively long pulse interval durations. This might be explained by assuming that when the opposite pulses are sufficiently far apart in time, they will behave as separate pulses, thus mimicking stimulation at higher frequencies.

The importance of the choice of stimulus parameters is further illustrated by the different results that were obtained after administration of several doses of apomorphine or haloperidol. The frequency-dependent circling behaviour was found to be more sensitive to drug administration than the current intensity-dependent circling. In the former kind of experiments a dose-dependent, biphasic response to apomorphine was observed. In the latter experiments only higher doses of apomorphine influenced the circling intensity. Though haloperidol inhibited the circling intensity in both kinds of experiment, it was also more effective in frequency-dependent circling behaviour.

The low doses apomorphine that attenuated frequency-dependent contraversive circling also produced a decreased spontaneous locomotor activity (data not shown). This hypokinesia does not account for the depression in circling behaviour since a dose of apomorphine still producing sedation (0.2 mg/kg did not affect the circling intensity. These

sedative effects of apomorphine might be due to activation of presynaptically located dopamine receptors, controlling the synthesis and release of the transmitter [7, 10, 12]. Only the autoreceptors located on the dopamine nerve terminals are involved in the observed effect of low doses of apomorphine, since the applied electrical stimulation overcomes the effect of activation of receptors on the dopamine cell soma and dendrities. The above described decreased circling rate might thus be caused by an attenuation of the dopamine release resulting in a decreased imbalance in dopaminergic transmission.

Higher, stimulating doses of apomorphine, that activate postsynaptic dopamine receptors in the brain [1,14], inhibit both the current intensity- and frequency-dependent contraversive circling behaviour. This decreased efficacy of the electrical stimulation-induced circling after administration of higher doses of apomorphine is probably the result of a relatively smaller contribution of the increased dopamine release to an already strongly activated postsynaptic receptor population. This is confirmed by the finding that high doses of amphetamine ($\geq$5 mg/kg) also inhibit circling behaviour (in preparation). These effects of apomorphine might also be explained by assuming a different influence of this compound on striatal and mesolimbic dopaminergic neurotransmission [8].

Haloperidol decreases circling intensity by blocking the dopamine receptors, thus antagonising the effect of an increased, electrical stimulation-induced dopamine release. Both the apomorphine-induced inhibition of frequency-dependent contraversive circling after high and low doses was reversed by a dose of haloperidol that itself did not affect the circling intensity. The effect of a high dose of apomorphine on current intensity-dependent circling was partially antagonised by haloperidol, whereas the decrease after the lowest effective dose of apomorphine was unaffected. Apparently, under these test conditions haloperidol is only partially able to antagonise the inhibition in circling behaviour at the dose tested.

In conclusion, the above described experiment show that concurrent activation of the nigrostriatal and mesolimbic dopaminergic pathways will produce contraversive circling behaviour, whereas separate activation of these pathways results in postural asymmetry and increased locomotor activity respectively. The choice of the stimulation parameters strongly determine the results that are obtained after the administration of drugs that interfere with the stimulated neurotransmitter systems.

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