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# Do Cholinomimetics Specifically Antagonize Rotational Behavior?

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WESTERMANN, K. H. *Do cholinomimetics specifically antagonize rotational behavior?* PHARMAC. BIOCHEM. BEHAV. 15(5) 687–690, 1981.—Asymmetries of body posture and movement (rotational behavior) following intracerebral application of dopamine (DA, 200 μg/2 μl) or carbachol (30 and 60 μg/2 μl) were diminished by systemically applied oxotremorine (0.5–1.0 mg/kg IP). The combined intracerebral injections of DA and carbachol show localization-specific effects in caudate nucleus, substantia nigra and globus pallidus. The experiments point to specific dopaminergic-cholinergic interaction in different brain regions. Systemically applied cholinomimetics exert a generally suppressive action on motoric effects.

Rotational behavior  Cholinomimetics  Intracerebral injection  Dopaminergic-cholinergic interaction

IN animals with unilateral lesion of the nigrostriatal system [6, 15, 21], locus coeruleus [5], globus pallidus [12] or frontal cortex [2] the systemic application of substances with stimulating effects on central dopaminergic receptors causes asymmetries of posture and motion (rotational behavior). Asymmetries following lesions of the nigrostriatal system appear to be suppressed by cholinomimetics [8, 9, 13]. Dopaminergic-cholinergic interaction in the neostriatum has been demonstrated by various methods [1, 7, 11, 14], and a neostriatal mechanism of this nature might be involved in rotational behavior.

In earlier experiments we and other authors revealed that such interaction could also be mediated by the substantia nigra [9,18]. In the present study this hypothesis was tested using intracerebral injection of dopamine and carbachol. A further concern of this study was to test the specificity of cholinergic influences on rotational behavior. Asymmetries of posture and motion were examined under independent experimental conditions to aid in recognizing possible differences in the phenomena of asymmetry.

## METHOD

The subjects were female Wistar rats weighing 140–150 g. For intracerebral injection dopamine hydrochloride (DA) or carbachol were injected by microsyringe and a 0.3 mm Hamilton cannula into substantia nigra compacta (SN), nucleus caudatoputamen (NCP) and globus pallidus (GP) using a stereotaxic method [16] (coordinates according to König and Klippel [10]: SN: 2.2 mm ant.; 2.2 mm lat.; −2.5 mm vert., GP: 6.5 mm ant.; −0.5 mm vert.; 2.5 mm lat., NCP: 7.5 mm ant.; 2.5 mm lat.; +0.5 mm vert.). Intracerebral injections were performed on the right-hand side and each rat was used on one occasion only. Rotational behavior of rats was recorded in a spherical, automatically counting rotometer [15]. Full rotations towards each side were covered separately.

The total number of rotations during the observation period (60 min) was termed "activity."

$$\text{Activity} = \text{rotations towards the left} + \text{rotations towards the right side.}$$

The net rotational difference was calculated by determining the rotations in the dominant direction and then subtracting the rotations in the opposite direction. The value of this difference divided by activity was termed "dominance."

$$\text{Dominance} = \frac{\text{rotations towards the left} - \text{rotations towards the right side}}{\text{activity}}$$

Since we characterized movements towards the left side with the sign +, movements towards the right side with −, a dominance of +1.0 showed that all rotations were directed to the left side. Values of +0.5 or −0.5 indicated a small preference of one direction.

Body posture was observed for 30 min in an "open field system." For evaluation of asymmetry, a 4-point-graduation was used with the same prefixes (+=posture to the left; −=posture to the right side; for details see Costall and Naylor [3]). After the experiments the exact site of injection was verified histologically on cryostat sections from formaldehyde-fixed brains (Nissl-staining).

## RESULTS

Both rotations towards the left side (following DA-injection into the right SN or NCP) and rotations towards the right side (after DA-injection into the right GP) were reduced dose-dependently by systemically applied oxotremorine (Table 1). Investigations into the specificity of the observed DA effects (dose dependence, effects of haloperidol pretreatment or of saline injections) are dealt with elsewhere [17,18].

TABLE 1

EFFECT OF OXOTREMORINE ON CIRCLING INDUCED BY INJECTION OF DOPAMINE (DA; 200 μg/2 μl) INTO BRAIN REGIONS OF THE RIGHT SIDE

| DA-injection into | Dose of oxotremorine (mg/kg) | Rotational behavior activity† | dominance |
|---|---|---|---|
| Substantia nigra | — | 138 ± 18 (80) | +0.86 |
| | 0.5 | 7 ± 3* ( 6) | +0.71 |
| | 1.0 | 8 ± 3* ( 6) | +0.50 |
| Nucleus caudatoputamen | — | 44 ± 8 (30) | +0.97 |
| | 0.5 | 46 ± 17 ( 7) | +0.91 |
| | 1.0 | 11 ± 3* ( 7) | +0.27 |
| Globus pallidus | — | 31 ± 5 (18) | −1.00 |
| | 0.5 | 33 ± 10 ( 5) | −0.91 |
| | 1.0 | 9 ± 2* ( 9) | −0.45 |

Number of animals in parentheses.
*$p<0.01$ calculated by Student's $t$-test.
† Mean number of turns/60 min ± S.E.M.

TABLE 2

ASYMMETRY OF POSTURE AFTER INTRACEREBRAL INJECTION OF DA FOLLOWING PRETREATMENT WITH OXOTREMORINE (1 mg/kg IP, 5 MIN BEFORE DA) OR ATROPINE (4 mg/kg IP, 15 MIN BEFORE DA)

| DA-injection into | Pretreatment | Asymmetry‡ | n |
|---|---|---|---|
| Substantia nigra | — | +5.5 ± 0.8 | 11 |
| | oxotremorine | +3.0 ± 0.7* | 11 |
| | atropine | +6.3 ± 1.7 n.s. | 7 |
| Globus pallidus | — | −5.5 ± 0.8 | 11 |
| | oxotremorine | −3.1 ± 1.1* | 11 |
| | atropine | −8.4 ± 0.7† | 5 |

*$p<0.05$, †$p<0.02$ compared to the effect of DA.
‡Mean rated score/30 min ±S.E.M.

Only after injections of DA into SN or GP was a temporary asymmetry of body posture observed. While the systemic application of oxotremorine diminished these asymmetries, preinjection with atropine had the opposite effect (Table 2).

Following intracerebral injection of carbachol, the activity and dominance of rotation following application of DA into any one of the three brain structures was influenced in various ways. Carbachol produced side-specific effects which were directed ipsilaterally after nigral injections and contralaterally after striatal injections (Fig. 1; 2 and 3). Few dominant rotations were observed following pallidal injection of carbachol. After carbachol-DA-combination into the SN, less activity to the left was apparent, whereas the activity caused by NCP injection was greater in comparison with the values after DA only (Fig. 1; 4 and 5). In GP the combination of drugs changed the dominance only, whereas the total number of rotations remained unchanged. In control experiments with saline (2 μl) or lower doses of carbachol (below 10 μg), we did not find any change in the specific effects of intracerebrally applied DA. In SN the injection of the high dose of carbachol was accompanied by stronger side effects (occasional loss of righting reflexes, dyspnea, tonic-clonic seizures) than in NCP and GP.

Surprisingly, the effects of topically applied carbachol on posture and rotational behavior were restricted or eliminated by preceding injection of oxotremorine. This attenuation was evident in all cases (Table 3).

## DISCUSSION

The influence of the nigro-neostriatal system on motor function and regulation of muscle tone is controlled by a dopaminergic-cholinergic-gabaergic loop between the striatum and substantia nigra [8, 11, 15]. In the striatum dopaminergic agonists and antagonists affect level, release and turnover of acetylcholine [14] and vice versa [1]. The therapeutic effectiveness of both antagonists of acetycholine and agonists of DA in the treatment of Parkinson's disease has also lead to the assumption of two opposing systems, the

TABLE 3

EFFECTS OF PRETREATMENT WITH OXOTREMORINE (1 mg/kg IP, 5 MIN) ON ASYMMETRIES EVOKED BY LOCAL INJECTION OF CARBACHOL (μg/2 μl) INTO SUBSTANTIA NIGRA (SN) OR NUCLEUS CAUDATOPUTAMEN (NCP)

| | Pretreament | | Inj. of carbachol into | |
|---|---|---|---|---|
| | oxotremorine | carbachol | NCP | SN |
| Asymmetry of | — | 30 | +3.5 ± 0.5 | −4.8 ± 0.3 |
| posture | 1 | 30 | +0.6 ± 0.4* | −3.2 ± 0.9 n.s. |
| Rotational | — | 60 | + 61 ± 18 | −23 ± 7 |
| behaviour | 1 | 60 | + 17 ± 7 n.s. | 0 ± 3* |

*$p<0.02$ compared to the effect of carbachol alone.

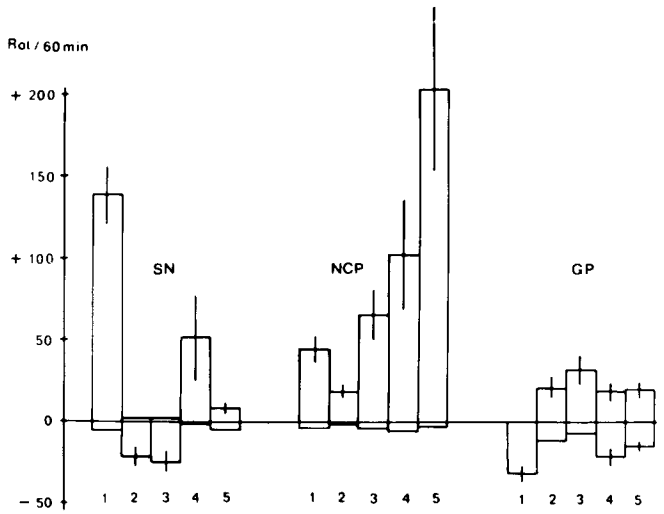


FIG. 1. Rotational behavior after unilateral injection into substantia nigra (SN), striatum (NCP) or globus pallidus (GP) of 1: DA (200 μg/2 μl); 2: carbachol (30 μg/2 μl); 3: carbachol (60 μg/2 μl); 4: DA + carbachol (200 μg + 30 μg); or 5: DA + carbachol (200 μg + 60 μg). All injections were performed on the right side. In combinations carbachol was applied 5 min before DA. +=rotations towards the left side; −=rotations towards the right side during 60 min.

balanced operation of which is necessary for the normal function of the basal ganglia [7,20].

In contrast to these biochemical and clinical findings, several behavioral experiments have shown synergistic rather than antagonistic effects of DA and cholinomimetics at the level of striatum, while antagonistic effects of both substances have been found at the level of SN [19,20] and nucleus accumbens [4]. The present results support these observations and reveal clear-cut differences between the actions of systemically and topically applied cholinomimetics.

Whereas systemic injection of oxotremorine reduced activity and dominance after intracerebral injection of DA independent of the intended direction, intracerebrally injected carbachol induced rotations of specific direction. The inhibition of the movements and asymmetries that appear following injection of DA (Table 1) or carbachol (Table 3) into one of the investigated areas by intraperitoneally applied oxotremorine leads us to propose a modulating cholinergic influence on a terminal target point in the control of movement. The hypothesis of an inhibitory cholinergic link is compatible with the effects of atropine on body posture and may explain the potentiating action of cholinolytics on asymmetries [8,13].

More specific motoric effects were found following intracerebral injection of carbachol. This drug antagonized the effects of dopamine in the SN, and enhanced its effects in the NCP. Such well-defined effects were not observed in the GP. These results support the publications of Wolfarth [19,20]. An explanation as to whether the effects are mediated by intrinsic cholinergic systems or systems extrinsic to the substantia nigra and nucleus caudatoputamen cannot yet be given.

In conclusion, the above results suggest that (1) behavioral experiments in part provide additional evidence of a cholinergic-dopaminergic interaction in brain nuclei that biochemical experiments have shown to have antagonistic cholinergic-dopaminergic mechanisms [1,7]; (2) cholinomimetics exert specific effects on posture and movements only after intracerebral injection; (3) the dopaminergic-cholinergic interaction varies according to specific brain regions.

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