Physiological significance of dopamine autoreceptors as studied following their selective blockade by molindone

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Dopamine receptors are localized both on the postsynaptic effector cells and on the presynaptic dopamine neurons. The latter receptors are called dopamine autoreceptors (Carlsson 1975) and they seem to occur both on the nerve terminals (presynaptic dopamine receptors) and in the cell body region (Kehr et al 1972a; Roth et al 1973). The physiological significance of the dopamine autoreceptors should be revealed following selective blockade of these receptors. In the present work, molindone (3-ethyl-6,7-dihydro-2-methyl-5-morpholinomethylindole-4-(5H)-one) was found to produce such an effect resulting in a facilitation of the dopamine neurotransmission.

Male Sprague-Dawley rats, 185–290 g were used. Drugs given were: apomorphine HCl·1/2 H_2O (Sandoz, Basle), γ-butyrolactone (Merck, Darmstadt), 3-hydroxybenzylhydrazine HCl (NSD 1015; Per Lindberg, Department of Pharmacology, University of Göteborg), molindone HCl (Pharmacia, Uppsala), reserpine (CIBA-Geigy, Mölndal). Reserpine was dissolved in a few drops of glacial acetic acid and 5·5% glucose was added to the volume. The other substances were dissolved in 0·9% NaCl. The doses are presented for the forms indicated.

The presynaptic dopamine receptor activity was determined following complete cessation of the nerve impulse flow by γ-butyrolactone (750 mg kg^{-1}, i.p., 35 min). This treatment causes an enhancement of the tyrosine hydroxylase activity of the dopamine nerve terminals, in all probability due to reduced stimulation of dopamine receptors on the dopamine nerve terminals since the effect is abolished by apomorphine (Kehr et al 1972a; Roth, et al 1973; Walters & Roth, 1976). The peak effect of apomorphine was seen after about 2 mg kg^{-1}, i.p. (data not shown).

The tyrosine hydroxylase activity of the dopamine neurons in the corpus striatum was studied in vivo by means of the dopa accumulation following inhibition of the dopa decarboxylase by NSD 1015 (100 mg kg^{-1}, i.p., 30 min) (Carlsson et al 1972). The rats were killed by thoracotomy and exsanguination when anaesthetized with dichloromethane. The corpora striata were rapidly removed and homogenized in 0·4 M perchloric acid. The dopa was determined spectrofluorimetrically following cation exchange chromatography and oxidation (Kehr et al 1972b).

The postsynaptic dopamine receptor activity was determined by means of the apomorphine-induced rotation of rats in which the dopamine stores were depleted by reserpine (10 mg kg^{-1}, i.p., 4 h) and in which the corpus striatum was inactivated unilaterally by intrastriatal injection of 1 μl 25% KCl. The hypertonic KCl solution might inactivate the cells in the corpus striatum by persistent depolarization (Stock et al 1973). An equimolar amount of NaCl (1 μl, 20%) was simultaneously administered in the contralateral corpus striatum. The KCl and the NaCl were given by the help of guide cannulae implanted stereotaxically one or two days before the experiment. The coordinates of the tips of the injection cannulae were A 7·9, L 2·4, V + 0·5 according to the atlas of König & Klippel (1963). Immediately after the completion of the intrastriatal injections, apomorphine (2 mg kg^{-1}, s.c.) was given and the rats were placed in plastic cylinders with a diameter of 50 cm. The number of complete circles around a vertical axis to the KCl- or to the NaCl-treated side was counted for 60 min. The apomorphine-induced rotation of the rats with the corpus striatum inactivated on one side is probably caused by asymmetry of muscle tone plus increase in motor activity due to stimulation of postsynaptic dopamine receptors in the corpus striatum and in the nucleus accumbens, respectively (Andén 1976). The apomorphine-induced rotation was maximal following approximately 2 mg kg^{-1}, s.c. (data not shown).

The physiological significance of the dopamine autoreceptors was studied in rotation experiments as described above, apart from the treatments with reserpine and apomorphine.

The accumulation of dopa following decarboxylase inhibition was enhanced by γ-butyrolactone (see legend of Fig. 1). This effect of γ-butyrolactone was

Table 1. The influence of molindone on the rotational behaviour of rats following unilateral inactivation of the corpus striatum by means of 1 μl 25% KCl. An equimolar amount of 20% NaCl was given into the corpus striatum on the opposite side. KCl, NaCl and molindone were given just before the start of the recording. The direction of turning is indicated as ipsilateral (+) or contralateral (−) to the KCl-treated side. The values are means ± s.e.m. with the number of animals in parentheses.

Molidone, mg kg^{-1} i.p.	Direction of turning	Number of circles/30 min	
A. 0	Contralateral	− 25±4·0	(8)
B. 0·25	Contralateral	− 13±8·2	(4)
C. 0·45	Ipsilateral	+ 51±20·8***	(4)
D. 0·80	Ipsilateral	+ 48±16·3***	(7)
E. 1·50	Contralateral	− 2±12·1	(8)
F. 5·00	Contralateral	− 23±17·0	(4)

*** $P < 0{\cdot}001$ in comparison with the control group A (Student's *t*-test).

* Correspondence.

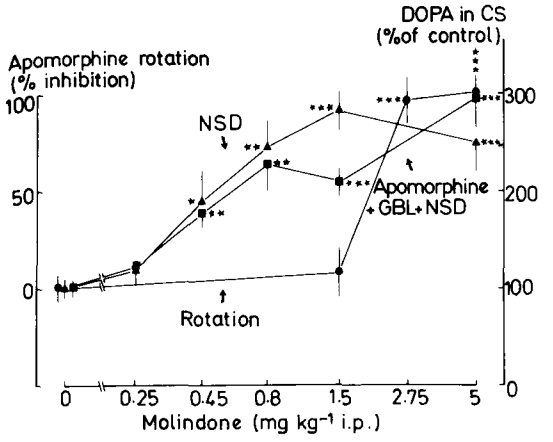

FIG. 1. Effects of different doses of molindone on the rotation induced by apomorphine and on the accumulation of DOPA in the corpus striatum (CS) following 3-hydroxybenzylhydrazine (NSD 1015). The rotation induced by apomorphine (2 mg kg^{-1} s.c., at time 0) was recorded in rats pretreated with reserpine (10 mg kg^{-1} i.p., 4 h) and in which the corpus striatum was inactivated unilaterally. The accumulation of dopa induced by NSD 1015 (100 mg kg^{-1} i.p., 30 min before death) was determined with and without apomorphine (2 mg kg^{-1} i.p., 40 min before death) plus γ-butyrolactone (GBL, 750 mg kg^{-1} i.p., 35 min before death). GBL alone increased the accumulation of dopa to $2{\cdot}41 \pm 0{\cdot}088$ $\mu g\ g^{-1}$ (n = 5). Molindone was given 15 min before the injection of apomorphine (rotation) or NSD (dopa accumulation). Control values: rotation 263 net circles to the inactivated side (n = 12), apomorphine + GBL + NSD 0·70 $\mu g\ g^{-1}$ dopa (n = 5), NSD 1·09 $\mu g\ g^{-1}$ dopa (n = 5). The number of experiments in the other groups was 4–8. The values are means ± s.e.m. Statistical significances (Student's *t*-test) for the differences from the controls are indicated by *** ($P < 0{\cdot}001$), ** ($P < 0{\cdot}01$) and * ($P < 0{\cdot}05$).

reversed by apomorphine (2 mg kg^{-1} i.p., 40 min), probably due to stimulation of presynaptic dopamine receptors. Molindone inhibited the effect of apomorphine on the synthesis of dopamine at doses of 0·45 mg kg^{-1} or higher (Fig. 1). The effect of apomorphine (2 mg kg^{-1} s.c.) on the rotation, due to stimulation of postsynaptic dopamine receptors, was inhibited by molindone only at a dose of 2·75 mg kg^{-1} or more (Fig. 1). From the ED50 values, molindone was about 4 times more potent in blocking the presynaptic than the postsynaptic effect of apomorphine. Molindone stimulated the NSD 1015-induced accumulation of dopa at doses similar to those blocking the presynaptic effect of apomorphine (Fig. 1).

The control rats not treated with reserpine and apomorphine rotated to the NaCl-treated side (Table 1). This effect might be the result of inactivation of the effector cells in the corpus striatum on the KCl-treated side. Molindone at doses of 0·45 and 0·8 mg kg^{-1} caused a marked rotation to the KCl-treated side. Increasing the dose to 5 mg kg^{-1} again induced a turning to the NaCl-treated side. These effects of molindone should be evoked from the NaCl-treated side since the corpus striatum was inactivated by KCl on the opposite side.

The results indicate that molindone is more potent in inhibiting the presynaptic than the postsynaptic dopamine receptors. In a previous study, molindone inhibited the presynaptic effect of apomorphine at a dose similar to that found in the present investigation (Walters & Roth 1976). In the same dose range, molindone also blocked the inhibitory effect of apomorphine on the dopamine receptors occurring on the cell bodies of the dopamine neurons in the substantia nigra and it increased the baseline firing rate of these dopamine cells (Bunney et al 1975). Thus, the dopamine autoreceptors on the cell bodies as well as on the nerve terminals appear to be more sensitive to molindone than the postsynaptic dopamine receptors on the effector cells.

By blocking the dopamine autoreceptors on the cell bodies and the nerve terminals of the dopamine neurons, molindone increases the firing rate and should facilitate the synthesis and the utilization of dopamine per nerve impulse in the nerve terminal area, respectively. These effects on the dopamine autoreceptors should result in an enhanced release of dopamine from the nerve terminals. If these actions occur without blockade of the postsynaptic dopamine receptors, molindone should produce signs of increased dopamine neurotransmission. Stimulation of the postsynaptic dopamine receptors in the corpus striatum on one side turns the heads and tails of rats to the contralateral side (Ungerstedt et al 1969). If this action is combined with a stimulation of postsynaptic dopamine receptors in the nucleus accumbens, the rats rotate to the contralateral side (Andén 1976).

In the present study, molindone at the doses of 0·45 and 0·8 mg kg^{-1} i.p. changed the rotation from the NaCl- to the KCl-treated side in the rats not treated with reserpine, i.e. a turning to the side on which the corpus striatum was inactivated. This finding indicates an increased dopamine neurotransmission in the corpus striatum on the NaCl-treated side following these doses of molindone. Since these doses were found to block the presynaptic but not the postsynaptic effect of apomorphine, it is likely that the enhanced dopamine neurotransmission is caused by inhibition of the negative feedback of the endogenous dopamine on the dopamine autoreceptors. This finding indicates that the autoreceptors are of importance physiologically as well as pharmacologically.

At high doses, molindone acts similarly to other neuroleptic drugs both in clinical and in animal investigations, probably due to blockade of postsynaptic dopamine receptors. At moderate doses, however, molindone has been reported to potentiate effects of dopa in rats and to induce euphoria in man (Rubin et al 1967; Sugerman & Herrmann 1967). It remains to be

clarified if the latter actions are caused by selective blockade of dopamine autoreceptors.

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The depressor and renal vasodilator responses to dopamine in the rat do not depend on prostaglandin biosynthesis

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The actions of many hormones are mediated (or moderated) by the stimulation of prostaglandin (PG) biosynthesis within the target tissue (Horton 1974). It is possible that the renal actions of dopamine (DA) in the rat might depend upon intrarenal PG synthesis. DA can have renal vasodilator actions in the rat (Chapman et al 1980a), and a number of PG's can cause renal vasodilatation (see review, Tobian 1976). DA causes diuresis and natriuresis (Chapman et al 1980b) and many PG's can similarly induce diuresis and natriuresis (Gross & Bartter 1973). Furthermore it has been asserted that some of the vascular actions of DA in the rat do depend upon local PG production (Chevillard et al 1978), and it is known that the renal actions of another catecholamine, noradrenaline, can involve the renal production of PG's (Dunham & Zimmerman 1970).

DA acts on three types of vascular receptors in the dog and man (Goldberg 1972) and in the rat (Chapman et al 1980a). Thus α-adrenoceptors mediate the vasoconstrictor, pressor responses, while β-adrenoceptors and specific dopamine receptors mediate the vasodilator, depressor responses. As cited above, it has been reported that the renal actions of noradrenaline can include an α-adrenoceptor-mediated stimulation of PG synthesis. We therefore decided to measure the vasodilator and vasodepressor actions of DA on the mean arterial blood pressure (MABP) and the renal blood flow of the rat, in the presence and absence of indomethacin (an inhibitor of endogenous prostaglandin production).

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Male Wistar albino rats, 300 g, were anaesthetized with sodium pentobarbitone and tracheostomized. A femoral artery was cannulated (Portex, PP25) and connected to a pressure transducer (Elcomatic, Glasgow) and pen recorder (Vitatron U.K. Ltd. Maidenhead) for the measurement of mean arterial blood pressure (MABP). A femoral vein was cannulated (Portex, PP25) for the intravenous infusion of drugs.

The surface of the left kidney was exposed with the aid of retractors, and two H_2-sensitive Pt electrodes were inserted to different depths within the kidney parenchyma. These two electrodes were used to measure H_2 concentrations (see Haining & Turner 1966) so that blood flows could be calculated for two highly localized regions of the kidney. Calibration marks were made on the electrodes so that one electrode could be inserted to a depth of 1 mm for the measurement of cortical blood flow, and the second electrode could be inserted to a depth of 2 mm from the kidney surface for the measurement of outer medullary blood flow. A single calomel–KCl bridge, held against the exposed tissues of the leg, completed the two circuits. The preparation was then left 30 min for renal function to stabilize.

Small volumes of H_2 gas were introduced into the tracheal cannula for one or two inspirations and distributed via the blood supply to the tissues of the body. Dissolved H_2, detected by the Pt electrodes in the kidney, caused a change in electrical current through the electrode and recording system; this current was recorded on a Vitatron logarithmic recorder. The rate of change of the renal $[H_2]$ was used to calculate RBF. This method for