Effect of ergot drugs on central catecholamine neurons: evidence for a stimulation of central dopamine neurons

In our studies on the neuroendocrine role of the tubero-infundibular dopamine neurons (Fuxe & Hökfelt, 1970; Hökfelt & Fuxe, 1972a, b) ergocornine and 2-Br-α-ergocryptine (CB154) were used as tools to inhibit prolactin secretion (see Flückiger & Wagner, 1968). It was discovered that both drugs exerted dopamine-like activity in the neostriatum probably mainly by directly stimulating dopamine receptors in the telencephalic areas. Chemical and functional studies have now been made to evaluate the effects of the two ergot drugs on dopamine and noradrenaline mechanisms.

Male Sprague-Dawley rats (150–200 g) were fed freely on a semi-synthetic diet under a standard light-dark schedule (light on at 6 a.m. and off at 8 p.m.).

Catecholamine turnover. The tyrosine hydroxylase inhibitor α-methyltyrosine-methylester (H44/68) was used (Andén, Corrodi & Fuxe, 1969). A difference in the extent of catecholamine depletion after treatment with the ergot drugs compared with controls gives an indication of a change in amine turnover. The dopamine and noradrenaline contents in brain were determined both biochemically (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962) and histochemically (Falck, Hillarp & others, 1962; Hillarp, Fuxe & Dahlström, 1966). The fluorescence intensity of the neostriatal and limbic dopamine nerve terminals and the hypothalamic and cortical noradrenaline nerve terminals was evaluated semi-quantitatively on coded slides by two investigators. Doses of 1–10 mg kg^{-1} of ergocornine-hydrogen-maleinate and CB154-mesylate were studied using intraperitoneal injections. The doses refer to the base. The dose of H44/68 was 250 mg kg^{-1} (i.p.) given 4 h before killing. For further details see Table 1.

Studies on rats with a unilateral 6-OH-DA-induced degeneration of the nigro-neostriatal dopamine pathway. 6-Hydroxydopamine (6-OH-DA) (8 μg per 4 μl) was slowly infused into the substantia nigra according to Ungerstedt (1968). The dopamine receptors in the denervated neostriatum became supersensitive (Ungerstedt, 1971), and as a result the rats turned vigorously towards the innervated side after treatment with low doses of the dopamine receptor stimulating drug apomorphine (Ernst, 1967; Andén, Fuxe & others, 1967). After treatment with a dopamine releasing agent such as amphetamine, on the other hand, the rats turned towards the denervated side, since no release of dopamine can occur on this side. The turning of 6 animals which had previously been tested and showed a response to apomorphine in as low doses as 0·25 mg kg^{-1}, was registered quantitatively via a microswitch on an electromagnetic counter (Ungerstedt & Arbuthnott, 1970).

The flexor hindlimb reflex is dependent on noradrenaline receptor activity (Carlsson Magnusson & Rosengren, 1963; Andén, Jukes & Lundberg, 1966) and dopa causes an increase in this reflex after monoamine oxidase inhibition. Whether ergocornine and CB154 could block this increase in flexor reflex activity in doses of 5–10 mg kg^{-1} (i.p.) was tested. Nialamide (100 mg kg^{-1}, i.p.) was given 1 h after spinal cord transection and 3 h before L-dopa (10 mg kg^{-1}, i.p.). The ergot drugs were given 1–2 h before L-dopa injection. The strength of the flexor reflex was semiquantitatively estimated on coded animals. 4–5 animals were studied with each dose.

Whether like apomorphine (Andén & others, 1967) ergocornine (10 mg kg^{-1}) and CB154 (20–50 mg kg^{-1}) could cause behavioural activity in animals pretreated with reserpine (10 mg kg^{-1}, i.p., 20 h before testing) and H44/68 (200 mg kg^{-1}, i.p., 2 h before testing) was examined in 3 animals per dose.

Catecholamine turnover results are summarized in Table 1. The levels of dopamine in rat brain were not affected by the ergot drugs at 5 mg kg^{-1}, but this dose caused a

Table 1. *The effect of 2-brom-α-ergocryptine and ergocornine on the H44/68 induced dopamine and noradrenaline depletion in the rat brain.* The ergot drugs were given i.p. 15 min before the H44/68 injection (250 mg kg^{-1}, i.p., 4 h before killing). The values are given as % of normal values (noradrenaline = 367 ± 24 ng g^{-1}; dopamine = 602 ± 16 ng g^{-1}; n = 5). n = number of experiments. Statistical significance calculated according to Student's *t*-test. CB154 = 2-brom-α-ergocryptine.

	Dose	n	Noradrenaline	Dopamine	Statistical significance	
Normal	mg kg^{-1}	5	100 ± 6·5 (*e*)	100 ± 2·6 (*a*)		
CB154 ..	5	4	79 ± 7·6 (*f*)	105 ± 5·3	(*e*)–(*f*) *P*	0·1
Ergocornine ..	5	4	64 ± 2·7 (*g*)	91 ± 2·3	(*e*)–(*g*) *P*	0·005
H44/68 ..		20	48 ± 1·4 (*h*)	30 ± 0·8 (*b*)	(*b*)–(*c*) *P*	0·001
					(*b*)–(*d*) *P*	0·001
CB154 ..	5	4	32 ± 2·5 (*i*)	45 ± 3·8 (*c*)	(*h*)–(*i*) *P*	0·001
Ergocornine ..	5	4	27 ± 1·6 (*j*)	59 ± 3·3 (*d*)	(*h*)–(*j*) *P*	0·001

clearcut retardation of the disappearance of dopamine in the whole brain after H44/68. The histochemical studies on the neostriatal and limbic dopamine nerve terminals revealed a dose-dependent deceleration of disappearance of dopamine fluorescence in the neostriatum and the limbic forebrain after H44/68, (1–10 mg kg^{-1}, 2–6 animals at each dose). Brain noradrenaline levels were reduced by both drugs at 5 mg kg^{-1}. With this dose an acceleration of the disappearance of noradrenaline in whole brain was also observed after treatment with H44/68 (see Table 1). The histochemical results revealed a decrease in the fluorescence in the hypothalamic and cortical noradrenaline nerve terminals after ergocornine (5 mg kg^{-1}) as well as after CB154 (10 mg kg^{-1}). After H44/68 treatment ergocornine 5 mg kg^{-1} (i.p.), causes an increase in the fluorescence disappearance of from the cortical and hypothalamic noradrenaline nerve terminals.

Results from rats with a unilateral 6-OH-DA induced lesion of the nigroneostriatal dopamine pathway. Both ergocornine and CB154 mimicked the action of apomorphine. The threshold doses for onset of turning behaviour towards the innervated side were 0·25 — 0·5 mg kg^{-1}. With a dose of 5 mg kg^{-1} the total number of turns varied mainly from 2000 to 4000 and the duration of action after this dose was 5–10 h. The animals showed stereotyped sniffing behaviour while turning. The ergocornine-induced turning behaviour started within 5 min, whereas the CB154-induced turning behaviour started only after 50–60 min. The effects of CB154 (5 mg kg^{-1}) were markedly reduced by pretreatment with the dopamine receptor blocking agent pimozide (1 mg kg,$^{-1}$, i.p., 1 h earlier).

In doses of 5–10 mg kg^{-1}, ergocornine caused a clearcut reduction of the dopa-induced increase in flexor reflex activity, whereas the effects of CB154 in the same dose range were slight or none.

In contrast to apomorphine (see Andén & others, 1967), CB154, even in doses of 50 mg kg^{-1}, did not cause any behavioural activation in rats pretreated with reserpine and H44/68. After ergocornine a behavioural response was present but consisted mainly of tremor and alternating movements of the head and forepaws together with a flat body posture without any clear locomotion.

Ergocornine and CB154 have been found to mimic the action of apomorphine both bio- and histochemically and functionally. Thus, these ergot drugs decrease in a

dose dependent way dopamine turnover in the neostriatum and the limbic system, since the H44/68-induced disappearance of dopamine in these areas is reduced by these drugs. This decrease is probably due to a dopamine receptor stimulating action, since both drugs mimic the action of apomorphine in rats with a unilateral 6-OH-DA-induced degeneration of the nigro-neostriatal dopamine pathway, and the response can be blocked by pimozide. In view of these findings ergocornine and CB154 may represent new types of dopamine receptor stimulating agents with a prolonged action in as low doses as apomorphine (0·25–1 mg kg^{-1}). However, in contrast to apomorphine these drugs appear to be relatively inactive in normal rats previously depleted of all known dopamine stores by reserpine and H44/68 pretreatment, whereas behavioural activation is observed in normal untreated rats.

The studies on noradrenaline neurons indicate that ergocornine, and maybe CB154, alone can cause a partial depletion of noradrenaline stores in the whole brain and can increase the H44/68 induced disappearance of noradrenaline in cortical and hypothalamic noradrenaline nerve terminals in doses of 5 mg kg^{-1}. These results can be explained on the basis that these drugs have a weak reserpine-like action on noradrenaline neurons. Thus, in noradrenaline depletion induced by inhibition of the amine's synthesis is mainly due to release by nerve impulses whereas that induced by a reserpine-like drug is mainly due to increased intraneuronal breakdown by monoamine oxidase. It may be, however, that the noradrenaline receptor blocking action of ergocornine could contribute to the acceleration of noradrenaline depletion by H44/68, since the dopa-induced increase in the flexor hindlimb reflex was reduced by ergocornine.

In conclusion, ergocornine and CB154 appear to be biologically very active drugs with several actions on brain dopamine and noradrenaline mechanisms. In addition, ergocornine has recently been found to be a 5-HT receptor stimulating agent (Corrodi, Fuxe & others, unpublished data). The present results underline the importance of actions on dopamine synapses in the neostriatum and the limbic forebrain. The main action appear to be a direct dopamine receptor stimulation of a long duration. Ergocornine and especially CB154 may therefore prove to be of value in the treatment of Parkinson's disease.

This work has been supported by a Grant from Ollie och Elof Ericssons Stiftelse. The drugs were generously supplied by Prof. E. Flückiger, Sandoz, Basle. The excellent technical assistance of Mrs. M. Baidins, Mrs. A. Eliason and Miss B. Hagman is gratefully acknowledged.

Department of Pharmacology, University of Göteborg, Göteborg, and Astra Pharmaceuticals AB, Södertälje, Sweden

H. CORRODI

Department of Histology, Karolinska Institutet, Stockholm, Sweden

K. FUXE
T. HÖKFELT
P. LIDBRINK
U. UNGERSTEDT

December 28, 1972

REFERENCES

ANDÉN, N.-E., CORRODI, H. & FUXE, K. (1969). In *Metabolism of Amines in the Brain*, pp. 38–47. Editor: Hooper, G. London: MacMillan.

ANDÉN, N.-E., FUXE, K., HÖKFELT, T. & RUBENSSON, A. (1967). *J. Pharm. Pharmac.*, **19**, 335–337.

ANDÉN, N.-E., JUKES, M. G. M. & LUNDBERG, A. (1966). *Acta physiol. scand.*, **67**, 387–397.

BERTLER, Å., CARLSSON, A. & ROSENGREN, E. (1958). *Ibid.*, **44**, 273–292.

CARLSSON, A. & LINDQVIST, M. (1962). *Ibid.*, **54**, 87–94.

CARLSSON, A., MAGNUSSON, T. & ROSENGREN, E. (1963). *Experientia*, **19**, 359–360.

CARLSSON, A. & WALDECK, B. (1958). *Acta physiol. scand.*, **44**, 293–298.
ERNST, A. M. (1967). *Psychopharmacologia*, **10**, 36–323.
FALCK, B., HILLARP, N.-Å., THIEME, G. & TORP, A. (1962). *J. Histochem. Cytochem.*, **10**, 348–354.
FLÜCKIGER, E. & WAGNER, H. R. (1968). *Experientia*, **24**, 1130–1131.
FUXE, K. & HÖKFELT, T. (1970). In *Aspects of Neuroendocrinology*, pp. 192–205. Editors: Bargmann, W. & Scharrer, B. Berlin-Heidelberg-New York: Springer-Verlag.
HILLARP, N.-Å., FUXE, K. & DAHLSTRÖM, A. (1966). In *Mechanisms of Release of Biogenic Amines*, pp. 31–37. Editors: Von Euler, U., Rosell, S. & Uvnäs, B. London: Pergamon Press.
HÖKFELT, T. & FUXE, K. (1972a). *Neuroendocrinology*, **9**, 100–122.
HÖKFELT, T. & FUXE, K. (1972b). In *Brain-Endocrine Interaction. Median Eminence: Structure and Function*, pp. 181–223. Basel: Karger.
UNGERSTEDT, U. (1968). *Eur. J. Pharmac.*, **5**, 107–110.
UNGERSTEDT, U. (1971). *Acta physiol. scand., Suppl.* 367, 69–93.
UNGERSTEDT, U. & ARBUTHNOTT, G. W. (1970). *Brain Res.*, **24**, 485–493.

Dopa reversal of hypoxia-induced disruption of the conditioned avoidance response

Catecholamines play a role in mediating certain behaviour under stimulus control. The conditioned avoidance response (CAR) is disrupted by administration of reserpine (Seiden & Carlsson, 1964), which blocks monoamine incorporation into storage granules (see Carlsson, 1966), or α-methyl-*p*-tyrosine (Corrodi & Hanson, 1966) which prevents catecholamine synthesis by inhibiting tyrosine hydroxylase (Nagatsu, Levett & Udenfriend, 1964). The administration of 3,4-dihydroxyphenylalanine (dopa) prevents the disruption of the CAR by these agents (Seiden & Carlsson, 1964; Corrodi & Hanson, 1966). If the metabolism of dopa is prevented by a peripherally acting dose of a decarboxylase inhibitor such as Ro 4-4602 (Bartholini & Pletscher, 1968), the reversal shown by dopa alone is enhanced; however, if a centrally active dose of the inhibitor is used, the administration of dopa is not effective (Seiden & Martin, 1971). These data indicate that the dopa reversal is centrally mediated by a metabolite of dopa—probably noradrenaline, dopamine, or both.

Exposure to hypoxia has been shown to disrupt behaviour in man and experimental animals (Birren, Fisher & others, 1946; Adler, Burkhardt & others, 1950; Vacher & Miller, 1968; Hurwitz, Robinson & Barofsky, 1971). In man, exposure to 14% oxygen has been reported to produce behavioural disturbances (Birren & others, 1946; Adler & others, 1950). At this oxygen concentration there is no apparent alteration in cerebral oxygen consumption, high energy phosphate production or oxidative carbohydrate metabolism (McIlwain, 1966). The synthesis of catecholamines, as well as indoleamines, is dependent on oxygen, however, and mild levels of hypoxia are associated with a decrease in rat brain tyrosine and tryptophan hydroxylase activity *in vivo* (Davis & Carlsson, 1973). Since the conditioned avoidance behaviour is influenced by alterations in catecholamine metabolism, the effect of hypoxia on this behaviour was examined.

Male Sprague-Dawley rats, 250 to 350 g, were tested under low oxygen conditions in a shuttle box. The CAR apparatus consisted of a two compartment chamber and the appropriate response was to move from one compartment to the other. A trial consisted of presentation of a buzzer, the conditioned stimulus, for 10 s followed by the presentation of intermittent shock, the unconditioned stimulus, in the presence of the conditioned stimulus. The 700 V shock was delivered for 0·5 s every 2·5 s through a grid floor over a resistance of 270 kohm. A trial and the stimuli terminated for 60 s when the animal moved to the other compartment. A response was con-