J. Pharm. Pharmac., 1973, **25**, 815–819 *Received February* 8, 1973

Evidence for catecholamine involvement in the suppression of locomotor activity due to hypoxia

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Locomotor activity of mice under 12 or 21% oxygen in nitrogen was measured. Under 12% oxygen there was a decrease in locomotor activity which was not reversed by administration of amphetamine; however, administration of a sub-threshold dose of L-dopa in combination with a peripheral decarboxylase inhibitor before the amphetamine restored the locomotor stimulant action of the drug under hypoxia. In contrast to amphetamine, the increase in locomotor activity after the administration of a combination of noradrenaline and dopamine receptor stimulating agents, clonidine plus apomorphine respectively, was not inhibited by hypoxic conditions. Data suggest an involvement of central catecholamine neurotransmission in the disruption of locomotor activity due to hypoxia.

Oxygen deprivation affects nervous tissue earlier and with a greater degree of severity than does any other tissue (see *e.g.* Foley, 1967) and induces behavioural symptoms in man and animals. Animals exposed to low oxygen display deterioration in open field behaviour (Hurder, 1951; Thompson & Pryer, 1956; Vacher & Miller, 1968) and operant behaviour (Seitz & Keller, 1940; Hurwitz, Robinson & Barofsky, 1971).

On the basis of gross observations Hurwitz & others (1971) suggested that brain catecholamines might be involved in the behavioural effects caused by hypoxia, although no correlation between hypoxia-induced changes in total brain catecholamine levels and in behaviour could be shown. Recently, however, Davis & Carlsson (1973) showed that hypoxia resulted in a decrease in the rate of tyrosine and tryptophan hydroxylation. This was measured by the accumulation of 3,4-dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP), respectively, after inhibition of aromatic amino-acid decarboxylase (see Carlsson, Davis & others, 1972). In addition, L-dopa was shown to reverse the hypoxia-induced suppression of a conditioned avoidance response (Brown, Davis & Carlsson, 1973).

We have now further examined the involvement of the catecholamines in hypoxia-induced alterations in locomotor activity. For this purpose, drugs with an indirect ((+)-amphetamine) or direct (apomorphine plus clonidine) action on the catecholamine receptors were used. The stimulant action of (+)-amphetamine can be prevented by pretreatment with the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (α-MT) (Randrup & Munkvad, 1966; Weissman, Koe & Tenen, 1966; Hanson, 1967; Dominic & Moore, 1969) and a subthreshold dose of L-dopa has been shown to restore the amphetamine-induced stimulation in α-MT-pretreated rats (Randrup & Munkvad, 1966; Hanson, 1967), thus implying that the action of (+)-amphetamine is indirect, being dependent on newly synthesized catecholamines. As oxygen deprivation reduces the rate of tyrosine hydroxylation (Davis & Carlsson, 1973) it would be expected that hypoxia would interfere with the (+)-amphetamine-induced locomotor stimulation and that the stimulatory effect of amphetamine could be restored by administration of L-dopa. As direct acting agents, the dopamine-receptor stimulating

agent apomorphine (Andén, Rubenson & others, 1967; Ernst, 1967) and the noradrenaline-receptor stimulant agent clonidine (Andén, Corrodi & others, 1970) were used. The combination of these two agents stimulates locomotor activity (Andén & others, 1970; Maj, Sowinska & others, 1972), an action which is not prevented by interference with catecholamine synthesis or storage (Andén, 1970), and so would not be expected to be antagonized by hypoxia.

METHODS

Subjects. Female mice weighing about 20 g of the N.M.R.I. strain (Anticimex, Stockholm) were used no earlier than one week after the arrival from the supplier. Mice were housed together under constant temperature and regulated light/dark conditions (light period was from 6.00 a.m. to 6.00 p.m.).

Drugs. The following drugs were used: (+)-amphetamine sulphate (Smith, Kline & French, Welwyn Garden City), L-3,4-dihydroxyphenylalanine methylester HCl (L-dopa, H 19/61, Hässle, Mölndal), N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine (Ro 4-4602, F. Hoffman-La Roche, Basle), apomorphine HCl·1/2 H_2O (Sandoz, Basle), clonidine HCl (Catapresan, Boehringer Ingelheim AB, Stockholm).

All the drugs were dissolved in 0·9% NaCl. The dose of L-dopa refers to the amino-acid. The dose of all other drugs was given in the forms indicated. All the injections were given intraperitoneally in a volume of 0·01 ml g^{-1} weight.

Apparatus. Locomotor activity was measured by means of a "M/P 40 Fc Electron Motility Meter" (Motron Products, Stockholm). The instrument was equipped with 40 photoconductive sensors arranged in 5 rows of 8 cells with a centre to centre distance of 4 cm. The cells were covered by a translucent plastic box, upon which was placed a transparent 6 litre plastic carrying cage (25 × 17 × 11 cm). This cage was made air-tight by a plastic lid containing inlet and outlet holes. The light source was a regular 45 watt lamp mounted 45 cm above the photoconductive sensors. Every 10th interruption of the beam was recorded by an external time controlled print out counter. The motility meter was housed in an air-conditioned, sound-attenuated chamber.

Low oxygen condition was produced by mixing oxygen and nitrogen. They were mixed after passing through 2 gas flow meters and the mixtures of 12 or 21% oxygen in nitrogen was then fed into the carrying cage at 4 litres min^{-1}.

Procedure. The locomotor activity of 3 mice/group was measured every 10 min for 60 min, starting 5 min after the animals were placed in the cage. The gas mixture was administered immediately after placing the mice in the apparatus. Pretreatment was with L-dopa (25 mg kg^{-1}) 20 min; (+)-amphetamine (3 mg kg^{-1}) or apomorphine plus clonidine (3 mg kg^{-1}) 5 min before the animals were placed in the cage. Since peripheral factors may contribute significantly to the overall behavioural effects of L-dopa (Butcher & Engel, 1969a,b), an inhibitor of the peripheral dopa decarboxylase, Ro 4–4602 (Bartholini, Burkard & others, 1967; Bartholini & Pletscher, 1968) was given in a dose of 50 mg kg^{-1} (i.p.), 30 min befor the injection of L-dopa or at a corresponding time interval. Control animals received 0·9% NaCl (0·01 ml g^{-1} weight) at corresponding time intervals. Treatments were given according to a modified Latin-square design and all groups were tested between 9.00 a.m. and 1.00 p.m. The number of groups used per treatment were between five and nine as indicated in Fig. 1.

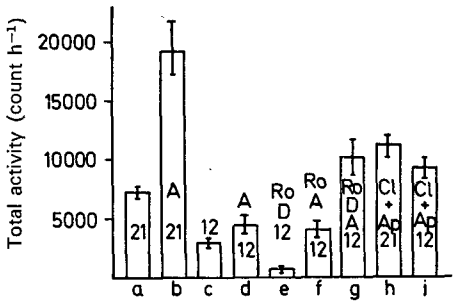

FIG. 1. The effect of oxygen deprivation on (+)-amphetamine- and apomorphine plus clonidine-induced locomotor stimulation. Mice in groups of three were injected with Ro 4-4602 (Ro, 50 mg kg^{-1}) 50 min; L-dopa (D, 25 mg kg^{-1}) 20 min, (+)-amphetamine (A, 3 mg kg^{-1}) or apomorphine (Ap, 3 mg kg^{-1}) plus clonidine (Cl, 3 mg kg^{-1}) 5 min before they were placed in the activity cage, immediately after which the gas mixture of 21 or 12% oxygen was administered. Control mice were injected with 0·9% NaCl (0·01 ml g^{-1}) at corresponding intervals. All injections were made i.p. The locomotor activity was measured for 60 min, starting 5 min after the animals were placed in the cages. The mean total counts of (N) groups ± s.e. are shown. N = 9 for all except groups e and f when N = 5.

Statistics. The results were statistically evaluted using one-way analysis of variance followed by *t*-test.

RESULTS

A. Total activity (Fig. 1)

In agreement with several reports (for review see van Rossum, 1970), the injection of (+)-amphetamine into mice in 21% oxygen (group b) caused a marked increase in total locomotor activity when compared to the 21% oxygen control group (group a) ($P < 0{\cdot}001$), but mice exposed to 12% oxygen (group c) showed a marked reduction in their locomotion compared to the 21% oxygen controls ($P < 0{\cdot}05$). When amphetamine was given to animals in 12% oxygen (group d), the low oxygen prevented the rise of motor activity seen in the 21% oxygen group receiving amphetamine (group b).

The combination of L-dopa plus Ro 4-4602, which when given together to mice in 12% oxygen (group e) did not cause an increase in locomotor activity, when given with amphetamine to animals in 12% oxygen (group g) produced a rise to about half the value found after amphetamine at 21% oxygen. At 12% oxygen, the administration of Ro 4-4602 and amphetamine without the L-dopa (group f) did not increase motor activity from that found at 12% oxygen alone (group c).

In contrast to amphetamine, the total locomotor activity observed after the administration of apomorphine plus clonidine was equal in 12 and 21% oxygen treated groups (h and i, $P > 0{\cdot}05$) and was greater than the respective control groups. The statistical significance between groups a and h as well as between groups c and i was less than 0·05.

B. Peak activity (Fig. 2)

The 10 min segment that showed the greatest number of counts for each treatment was plotted in order to compare the treatments. The administration of apomorphine plus clonidine to mice in either 12% (group g) or 21% oxygen (group f) produced the same maximal stimulation after amphetamine in mice exposed to 21% oxygen (group c) ($P > 0{\cdot}05$, c and f; $P > 0{\cdot}05$, groups c and g). The peak activity observed in animals receiving amphetamine under 12% oxygen was no greater than that in animals receiving 12% oxygen alone ($P > 0{\cdot}05$, groups b and d). The peak

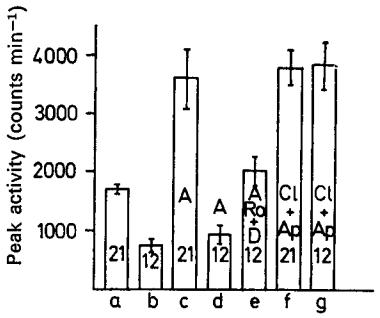

FIG. 2. Comparison of the maximal peak locomotor stimulation in mice treated with (+)-amphetamine or apomorphine plus clonidine under hypoxic conditions. For drug injection times see legend to Fig. 1. For each treatment, the 10 min segment which showed the greatest number of counts is given. The means of 9 groups ± s.e. are shown. Time of peak activities: a, b, g, 0–10 min. c, d, 30–40 min. e, 20–30 min. f, 10–20 min.

stimulation shown under 12% oxygen in animals receiving Ro 4-4602, L-dopa, and (+)-amphetamine (group e) was greater than in 12% oxygen controls ($P < 0{\cdot}05$). However, the peak activity of the mice given this latter treatment was no greater than the 21% control group ($P > 0{\cdot}05$, groups a and e).

DISCUSSION

In the present experiments the action of hypoxia like that reported for α-MT (for review see van Rossum, 1970) prevented the (+)-amphetamine-induced locomotor stimulation. Further, this effect of oxygen deprivation, like the effect of α-MT, was antagonized by a sub-threshold dose of L-dopa in combination with a peripheral dopa decarboxylase inhibitor. It is possible therefore, that the hypoxia-induced behavioural disruption is related to an interference with catecholamine synthesis. In support of this suggestion, Davis & Carlsson (1973) showed that oxygen deprivation results in a decrease in the rate of tyrosine hydroxylation.

Since Ro 4-4602 under 12% oxygen was unable to affect the locomotor stimulating action of (+)-amphetamine ($P > 0{\cdot}05$, groups d and f in Fig. 1), the enhancement of the amphetamine action seen after Ro 4-4602 plus L-dopa is in all probability due to the L-dopa. The finding that a small dose of L-dopa in combination with a peripheral decarboxylase inhibitor in 12% oxygen animals caused a slight but significant decrease in locomotor activity is in agreement with earlier results of Strömberg (1970), although the mechanism for this is unknown. Since the dose of Ro 4-4602 used is known to selectively inhibit extracerebral dopa decarboxylase (Bartholini & Pletscher, 1968, 1969), the dopa-induced restoration of the amphetamine locomotor stimulating activity obtained under 12% oxygen is in all probability due to the formation of catecholamines in the central nervous system.

The peak locomotor activity seen in mice under 12% oxygen treated with Ro 4-4602, L-dopa, and (+)-amphetamine did not reach the level of that observed after (+)-amphetamine under 21% oxygen. Since the dopamine-β-hydroxylase, which catalyses the conversion of dopamine to noradrenaline, is an oxygen-requiring enzyme, it may be that insufficient noradrenaline is formed to mediate a maximal (+)-amphetamine response. It may be that larger doses of L-dopa in combination with amphetamine would enhance the peak locomotor activity, but in the present investigation it was desirable to use a dose of L-dopa that had no stimulating effect by itself.

It can also be argued that the insufficiency of oxygen in muscle tissues may impair motor performance. However, under the hypoxic conditions in the present investigation this possibility seems less likely since direct activation of central catecholamine receptors by apomorphine plus clonidine resulted in the same total and peak activity under both 21 and 12% oxygen. The data do, however, support the suggestion that the hypoxia-induced impairment of the amphetamine-induced locomotor stimulation may be related to a presynaptic mode of action of oxygen deprivation, *e.g.* decreased rate of tyrosine hydroxylation.

In conclusion the present findings suggest that central catecholamines are involved in the behavioural effects observed under hypoxic conditions.

Acknowledgements

R.B. was supported by a post-doctoral fellowship from the Swedish State Medical Research Council. J.E. was supported by the Swedish Board for Technical Development.

This research was also sponsored by the Swedish Medical Research Council (grant No. B73-04X-2157-07), the Faculty of Medicine, University of Göteborg, and Magnus Bergvalls Stiftelse.

For a generous gift of drugs we are grateful to Hässle, Mölndal (L-dopa methylester HCl), F. Hoffman-La Roche, Basle (Ro 4–4602), Boehringer Ingelheim, Stockholm (clonidine).

The valuable technical assistance of Mr. Kenn Johannessen is gratefully acknowledged.

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