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Hypokinesia Produced by Anterolateral Hypothalamic 6-Hydroxydopamine Lesions and Its Reversal by Some Antiparkinson Drugs[1]

ROGER F. BUTTERWORTH, FERDINAND BÉLANGER AND ANDRÉ BARBEAU

Department of Neurobiology, Clinical Research Institute of Montreal, Montreal, Quebec, Canada

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BUTTERWORTH, R. F., F. BELANGER AND A. BARBEAU. *Hypokinesia produced by anterolateral hypothalamic 6-hydroxydopamine lesions and its reversal by some antiparkinson drugs.* PHARMAC. BIOCHEM. BEHAV. 8(1) 41–45, 1978. – Hypokinesia produced by stereotaxic microinjection of solutions of 6-hydroxydopamine into the anterolateral hypothalamus of male rats is accompanied by a generalized reduction in brain noradrenaline levels and a reduction of dopamine in the striatum and cerebral cortex. The hypokinesia is reversed by the putative dopamine-receptor agonists apomorphine, ET-495 and CB-154 as well as by the amino acids L-Dopa and m-tyrosine when administered in combination with the peripheral decarboxylase inhibitor Ro 4–4602. The relative importance of noradrenergic and dopaminergic systems in the mediation of the action of anti-akinesia drugs is discussed.

Hypokinesia 6-Hydroxydopamine Brain catecholamines Hypothalamus Anti-Parkinson drugs

A DECREASE in spontaneous locomotor activity, similar to hypokinesia, is known to result from hypothalamic lesions. Destructive lesions at the level of the dorsomedial nuclei and periventricular system of the hypothalamus, involving noradrenergic systems, result in a decrease in spontaneous locomotor activity without affecting the alertness of the animal [16]. Bilateral electrolytic lesions of the ascending dopaminergic fibres in the lateral hypothalamus have been reported to produce hypokinesia accompanied by mild catalepsy [5]. More recently, Smith and Young [18] proposed a model of hypokinesia produced by lesions in the anterolateral hypothalamus; these authors suggested that the model could be useful in the screening of potential antiparkinson drugs.

Experiments have been undertaken to evaluate this model of hypokinesia produced by microinjection of 6-hydroxydopamine into the anterolateral hypothalamus with particular reference to: (1) changes in dopamine (DA), noradrenaline (NA) and serotonin (5HT) concentrations in all regions of the brain of lesioned animals. (2) The ability to reverse this hypokinesia through the use of the potential anti-parkinson drugs apomorphine, ET-495 (Piribedil), CB-154 (Bromocriptine). L-Dopa, and the amino acid m-tyrosine, which has recently been shown to possess anti-parkinson-like activity in rats [20].

METHOD

Male, Sprague-Dawley rats weighing 240–300 g were used and were housed in our animal quarters under constant conditions of temperature, humidity and light cycles. Rats were anaesthetised with sodium pentobarbital (60 mg per kg, IP) and 4 μl of a solution of 6-hydroxydopamine (6.5 μg per μ in distilled water containing 0.4 μg ascorbic acid, was injected bilaterally into the hypothalamus according to the following coordinates as described by Smith and Young [18]: anterior 7.0 mm, lateral 2.0 mm, 8.0 mm down from the dura, based on DeGroot's stereotaxic [6]. A David Kopf stereotaxic frame (David Kopf Instruments Inc.) was used to support the animal. Microinjections were made at a rate of 1 μl per min., using a Hamilton No. 701, 10 μl syringe fitted with a shortened (3 cm) needle. Sham-operated rats received isovolumetric quantities of ascorbic acid in distilled water. Forty-eight hours following recovery from surgery, animals were introduced singly into a large wooden box (88 cm × 88 cm × 60 cm) the floor of which was marked into 16 squares of equal size. The box was illuminated evenly and placed in a minimum-noise environment. The number of squares entered per 2 min observation period was noted for each animal during a total observation period of 120 min. The effect of various drugs on locomotor activity was studied; results were expressed as the mean ± SE of at least 5 determinations per drug dose administered. Observations took place between 9:00 a.m. and 11:00 a.m. in all cases.

In a separate experiment, a series of 6 animals were subjected to the lesioning procedure and 2 days later

[1] Send reprint requests fo Dr. André Barbeau, M.D., Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7.

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examined for hypokinesia. The animals were sacrificed and their brains quickly removed and dissected on ice into the following regions: cerebral cortex, midbrain, hypothalamus, striatum, hippocampus, medulla oblongata and cerebellum. A series of 6 sham-operated animals received equivalent volumes of ascorbic acid solution as previously described. Brain regions were frozen in liquid N_2 until the monoamine estimations were carried out. Levels of DA, NA and 5HT were determined by the combined alumina absorption-spectrofluorometric method of Butterworth *et al.* [3].

The following drugs were used: L-3, 4-dihydroxyphenyl alanine (L-Dopa) (Calbiochem), Ro 4–4602 (benzerazide) was kindly supplied by Dr. J. Gareau, Hoffmann-LaRoche Inc., Montreal, ET-495 (Piribedil) (Servier Labs.), CB-154 methane sulphonate (Bromocriptine) (Sandoz Pharmaceuticals). Apomorphine hydrochloride was kindly supplied by Dr. André Clermont, Schering Corporation, Montreal. All drugs were administered in physiological saline with the exception of ET-495 which was dissolved in buffered saline as previously described [4]. 6-hydroxydopamine HBr and m-tyrosine were purchased from Sigma Chemicals, St. Louis, MO, U.S.A.

RESULTS

Effect of Hypothalamic 6-Hydroxydopamine Lesions on Concentration of DA, NA and 5HT in Regions of the Rat Brain

As is clearly seen from Fig. 1, bilateral 6-hydroxydopamine lesions at the level of the anterolateral hypothalamus caused significant reductions (up to 80%) of noradrenaline in the region dissected as hypothalamus according to the dissection guidelines of Glowinski and Iversen [9]. In only one region (hippocampus) was the reduction in noradrenaline not statistically significant, as analysed by unpaired student *t*-test.

Dopamine concentrations were found to be reduced by 50–60% in striatum and cerebral cortex but no change was observed in other regions normally found to contain measurable quantities of dopamine.

Serotonin levels remained unaffected by the bilateral lesion technique, with the exception of a small but significant decrease ($p \leq 0.02$) of 5HT concentration in the medulla oblongata.

Effect of 6-Hydroxydopamine Lesions on Spontaneous Locomotor Activity

Two days following recovery from bilateral hypothalamic lesions, animals were assessed for locomotor activity and the results are shown in Table 1.

Despite an otherwise normal and alert appearance, the rats showed a marked decrease in locomotor activity (Table 1), and frequently exhibited extension of the rear limbs in attempted movement. Lesioned rats suffered a mean weight loss during the 48 hr period of 20.6 g compared to a weight gain of 12.4 g in the sham-operated group.

Effect of Dopamine-Receptor Agonists on 6-Hydroxydopamine Hypokinesia

Administration of apomorphine (1 mg per kg, SC) to the bilaterally 6-hydroxydopamine-lesioned rats produced a rapid reversal of the hypokinesia as shown in Fig. 2. The maximum intensity of action was within 30 min of

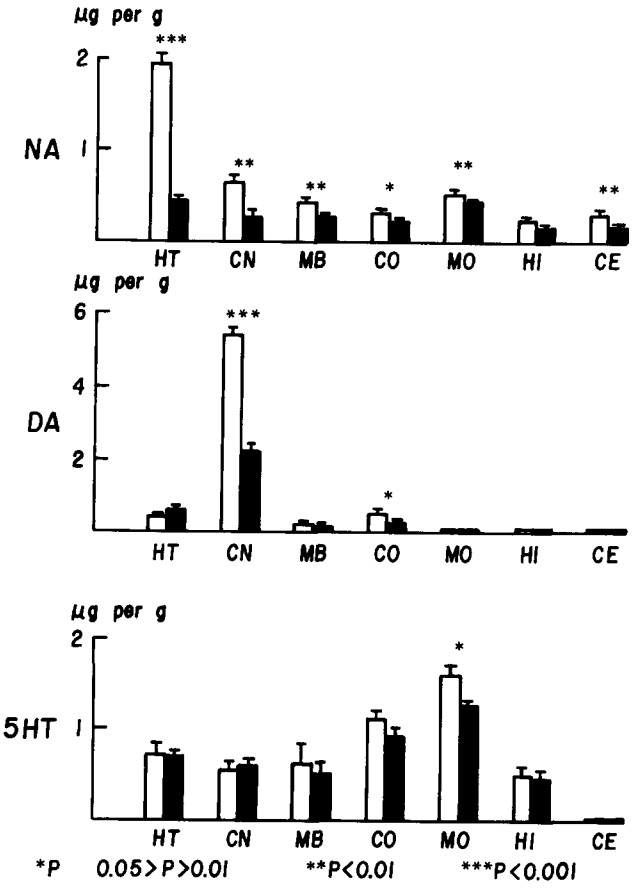

FIG. 1. Effect of 6-hydroxydopamine-induced hypothalamic lesions on cerebral monoamines. □ Sham operated rats; ■ 6-hydroxydopamine lesioned rats; HT: hypothalamus; CN: caudate nucleus; MB: midbrain; CO: cortex, MO: medulla oblongata, HI: hippocampus; CE: cerebellum. Histogram represents mean ± SEM, for a series of 6 rats.

administration of the drug but the duration of reversal of hypokinesia was short (of the order of 75 min); apomorphine's action paralleled brain accumulation of the drug as previously described [4]. Following administration of ET-495 (in doses of 30 mg and 75 mg per kg, IP), however, the duration of antihypokinetic action was markedly longer for both doses of ET-495 studied (Fig. 3). Bromocriptine (CB-154) when administered in a dose of 30 mg per kg, IP, caused a reversal of hypokinesia as shown in Fig. 4.

Effect of the Aminoacids L-Dopa and m-Tyrosine on 6 Hydroxydopamine Hypokinesia

Lesioned rats were treated with the peripheral L-Dopa decarboxylase inhibitor Ro 4–4602 (50 mg per kg, IP). This treatment alone has no effect on the hypokinesia, however, 20–30 min. following a subsequent dose of L-Dopa (66 mg per kg, IP) or m-tyrosine (100 mg per kg, IP) (Figs. 5 and 6) an intense, long-acting reversal of hypokinesia was apparent.

Effect of Other Anti-Parkinson Drugs

The following drugs, commonly used in an anti-Parkinson regimen when administered in the doses indi-

TABLE 1

EFFECT OF BILATERAL ANTEROLATERAL HYPOTHALAMIC 6-HYDROXYDOPAMINE LESIONS ON WEIGHT CHANGE AND LOCOMOTOR ACTIVITY

	6-Hydroxydopamine Lesioned Rats (N = 10)	Sham-operated Rats (N = 10)
Locomotor Activity (Total counts per 120 min) (Mean ± SE)	0.8 ± 0.1	24.3 ± 2.9
Weight Change (Mean ± SE)	Loss of 20.6 ± 3.1 g	Gain of 12.4 ± 1.0 g

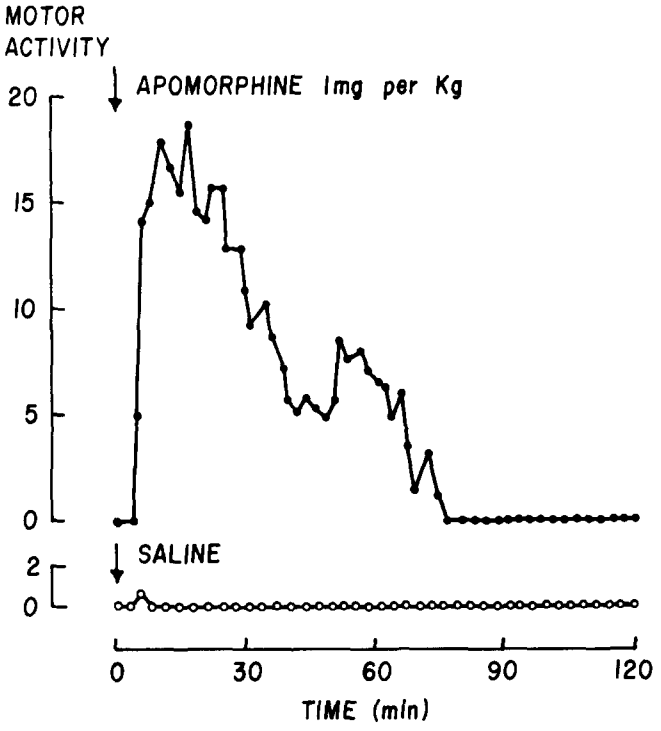

FIG. 2. Effect of apomorphine (1 mg per kg, SC) on 6-hydroxydopamine hypokinesia. Control animals received an equivalent volume of saline; each point represents the mean for a series of at least 6 rats. Motor activity was determined as described in section entitled METHOD.

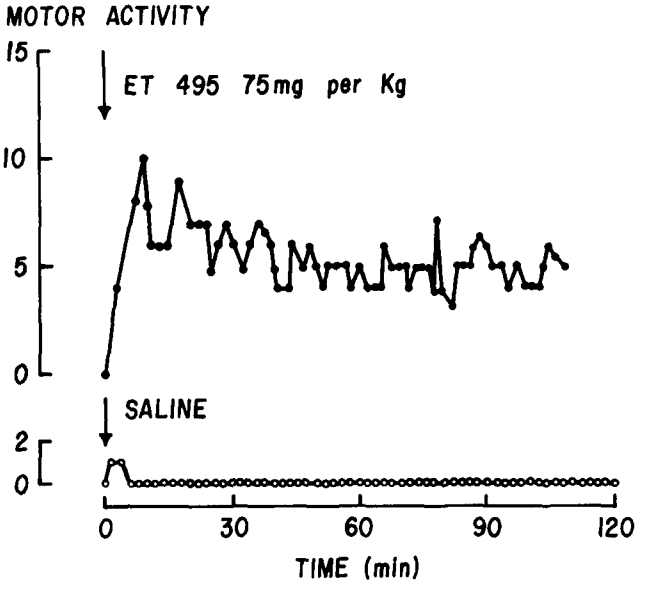

FIG. 3. Effect of Piribedil (ET-495) (75 mg per kg, IP) on 6-hydroxydopamine hypokinesia. Legend as described for Fig. 2.

cated, were found to be without effect on 6-hydroxydopamine induced hypokinesia: trihexyphenidyl, 3 mg per kg, IP; Amantadine HCl, 35 mg per kg, IP; SCH-15507 (Schering investigational drug), 100 mg per kg, IP; procyclidine HCl, 3 mg per kg, IP.

DISCUSSION

Following anterolateral hypothalamic injections of 6-hydroxydopamine, rats failed to move in an open field. In a previous report [7], in order to assess the pattern of catecholaminergic damage, forebrain lesions from rats lesioned as described were sectioned by vibratome and processed by the glyoxylic acid histofluorescence method. Catecholamine denervation was observed in lateral hypothalamus, anteromedial striatum, neocortex and limbic forebrain, leaving the catecholamine innervation to amygdala, thalamus, posterior striatum and brainstem intact. Biochemically, the changes in monoamine concentration (Fig. 1) following lesions in the anterolateral hypothalamus, resemble many of the monoamine changes found in post-mortem parkinsonian brain. While it is generally accepted that the major pathological change in Parkinson's disease is degeneration of the substantia nigra, other pigmented brain stem nuclei are also affected including the locus coeruleus, a major source of ascending noradrenergic fibres to the cerebrum, diencephalon and cerebellum [15]. Noradrenaline is reduced to 50% of normal levels in the hypothalamus, substantia nigra and inferior head of the caudate nucleus [11,17].

The putative dopamine receptor agonists, apomorphine, ET-495 and CB-154, were all shown to produce a reversal of the 6-hydroxydopamine-induced hypokinesia. This could be thought to occur by direct stimulation of dopamine receptors. However, Grabowska and co-workers [14] have shown that the locomotor stimulatory action of apomorphine is modified by several drugs known to affect both

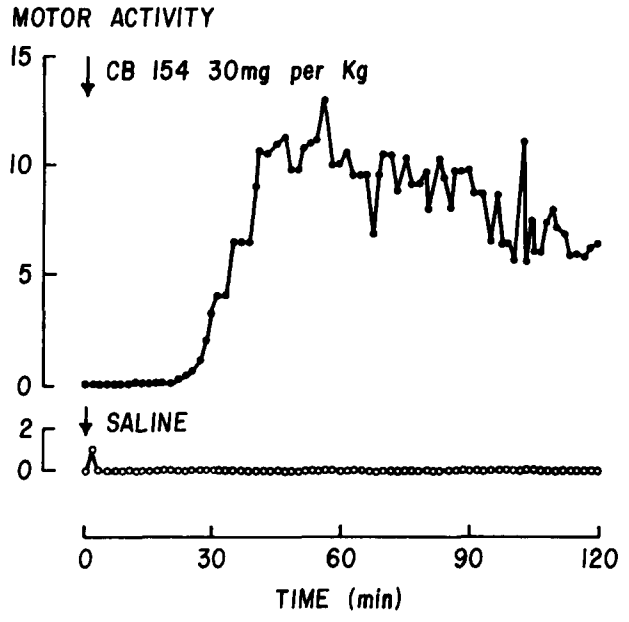

FIG. 4. Effect of Bromocriptine (CB-154) (30 mg per kg, IP) on 6-hydroxydopamine hypokinesia. Legend as described for Fig. 2.

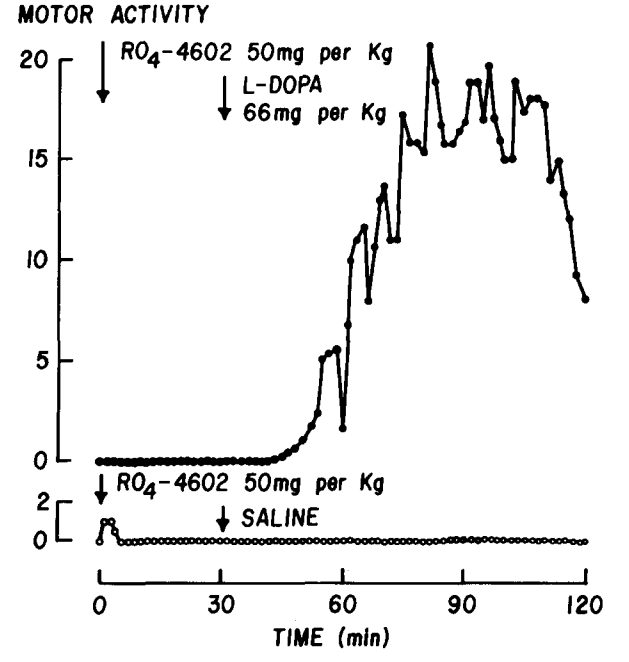

FIG. 5. Effect of L-DOPA (66 mg per kg, IP) on 6-hydroxydopamine hypokinesia. Legend as described for Fig. 2.

dopaminergic and noradrenergic systems. In addition, it has been reported that apomorphine's reversal of reserpine akinesia was increased two-fold by concurrent administration of clonidine, a drug believed to selectively stimulate central noradrenergic receptors. Clonidine alone was without effect, suggesting that both dopaminergic and noradrenergic stimulation appeared essential for reversal of reserpine-induced akinesia [15]. This supports the hypothesis of van Rossum and Hurkmans [21] that increases in motility are a result of stimulation of dopamine receptors but that noradrenaline plays an important modulatory role in this stimulation. The respective roles of dopamine, noradrenaline and serotonin in the pathophysiology of Parkinson's disease have been reviewed by Barbeau [1]. In addition, both apomorphine [2] and ET-495 [8] have been shown to cause changes in brain content of NA, casting doubts on the purely dopamine-receptor agonist mechanism proposed to explain their mechanism of action. Jenner and Marsden [12] have recently shown that reversal of reserpine-akinesia by ET-495 is accompanied by increased cerebral NA turnover due to a presynaptic action of NA neurons. Evidently, a more thorough study of the interaction of these potentially useful antiparkinson agents with brain monoamines is required before their mechanism of action is fully understood.

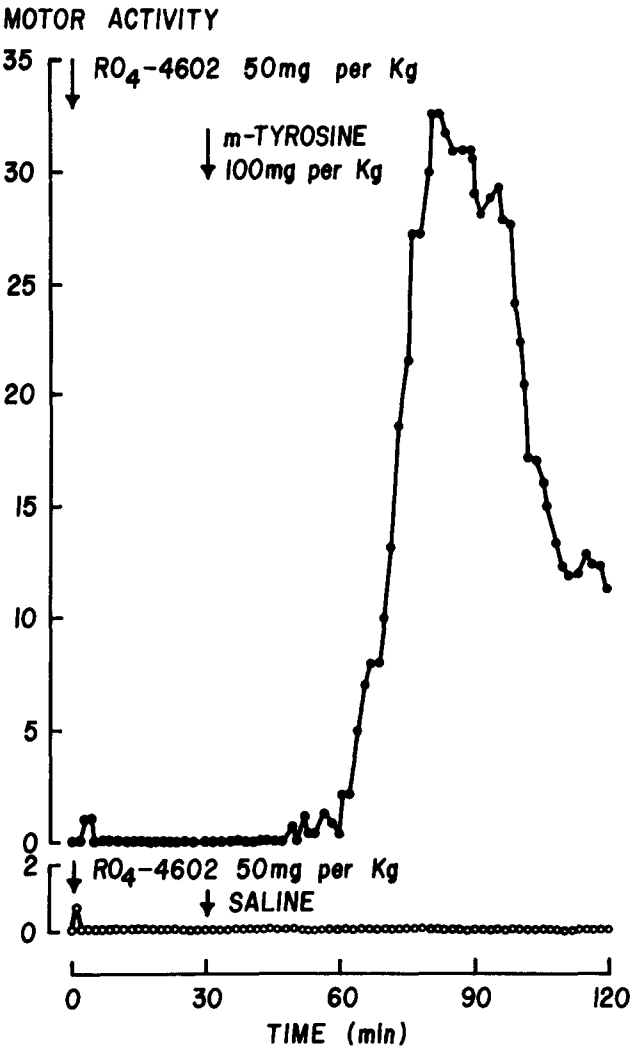

FIG. 6. Effect of m-tyrosine (100 mg per kg, IP) on 6-hydroxydopamine hypokinesia. Legend as described for Fig. 2.

The marked antagonistic effect of L-Dopa on 6-hydroxydopamine-induced hypokinesia represents further evidence for the mediation of the antiparkinson action of L-Dopa via both dopaminergic and noradrenergic systems.

Recently evidence has been presented to show that when L-Dopa is administered to animals, both the DA and NA produced are important for the parent amino acids' locomotor stimulatory and anti-akinesia effects [10,11]. In addition, L-Dopa has been shown to accelerate the turnover of NA in vivo [13].

M-tyrosine, administered in combination with the peripheral dopa-decarboxylase inhibitor Ro 4–4602, caused a long-acting reversal of hypokinesia (Fig. 6). It has been suggested by Ungerstedt *et al.* [20] that the mechanism of action of m-tyrosine is due to dopamine receptor stimulation. However, Smyth *et al.* [19] have recently presented evidence suggesting that m-tyrosine causes release of catecholamines, the mechanism of release involving m-tyramine, formed by decarboxylation of the parent m-tyrosine. The precise mechanism of action of m-tyrosine awaits a more complete study of its neurochemical action, including its effect on both DA and NA systems.

In conclusion, lesions of the anterolateral hypothalamus produce hypokinesia in experimental animals. This hypokinesia is accompanied by a selective decrease in cerebral DA and NA and is reversed by known anti-akinesia drugs. These results suggest a role for the known parkinsonian hypothalamic lesion in the pathophysiology of akinesia. The relative importance of dopaminergic and noradrenergic systems in the mechanism of action of anti-akinesia drugs remains to be established. Further work using the 6-hydroxydopamine lesion technique as an aid to a fuller understanding of the mechanism of action of potentially useful antiparkinson agents is presently underway.

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