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Effects of Nomifensine and Desipramine on the Sequelae of Intracerebrally-Injected 6-OHDA and 5,6-DHT

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WADDINGTON, J. L. *Effects of nomifensine and desipramine on the sequelae of intracerebrally-injected 6-OHDA and 5,6-DHT*. PHARMAC. BIOCHEM. BEHAV. 13(6) 915–917, 1980.—Pretreatment with either nomifensine or desipramine (15 mg/kg) attenuated the depletion of forebrain noradrenaline induced by unilateral 6-hydroxydopamine lesions of the medial forebrain bundle. Neither pretreatment influenced the depletions of striatal dopamine induced by medial forebrain bundle lesions with 6-hydroxydopamine or 5,6-dihydroxytryptamine, or contralateral rotational responses to apomorphine in the 6-hydroxydopamine-lesioned animals. Pretreatment with nomifensine attenuated the depletion of striatal dopamine induced by intrastriatal injections of 6-hydroxydopamine, consistent with inhibition of dopamine uptake *in vivo*. Nomifensine may differentially influence distinct axonal and terminal uptake mechanisms for dopamine.

Nomifensine Desipramine Dopamine uptake 6-Hydroxydopamine 5,6-Dihydroxytryptamine Rotational behaviour Antidepressants

NOMIFENSINE (NOM) is a novel tetrahydroisoquinoline antidepressant agent. It resembles tricyclic antidepressants such as desipramine in potently inhibiting *in vitro* re-uptake mechanisms for noradrenaline (NA) but, contrary to tricyclics, also markedly inhibits the re-uptake system for dopamine (DA) and exhibits DAergic activity in a variety of behavioural models. Its basic and clinical pharmacology has been the subject of recent extensive and substantive reviews [3,5]. The ability of pretreatment with NOM to attenuate the neurotoxic action of intraventricularly-administered 6-hydroxydopamine (6-OHDA) on NA and DA neurons, an effect believed to be initiated by incorporation of 6-OHDA into catecholamine neurons via specific uptake mechanisms, has been offered as evidence of inhibition of NA and DA uptake *in vivo* [6]. The present report describes the effects of pretreatment with NOM on the behavioural and neurochemical sequelae of both 6-OHDA and the indoleamine neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) administered intracerebrally onto the axons of monoamine neurons and into terminal regions of their efferent projections. This was to study (1) the effects of NOM on the action of monoamine neurotoxins applied at distinct positions on monoamine neurons to clarify its *in vivo* activity at these levels and (2) investigate the potential of NOM as a tool for improving the specificity of action of such neurotoxins. Comparisons were made with the effects of pretreatment with desipramine (DMI) which is, like NOM, a potent inhibitor of NA re-uptake but with minimal effects on DAergic processes [3,5].

METHOD

Male Sprague-Dawley rats of 150–200 g were anaesthetised with sodium pentobarbital (40–60 mg/kg IP) and were given unilateral lesions of the medial forebrain bundle (MFB) or of the striatum by intracerebral infusion of neurotoxins. Lesions of MFB were made with 8 μg 6-OHDA (as the hydrobromide, Sigma) in 4 μl saline containing 1 mg/ml ascorbic acid as antioxidant, or with 5 μg 5,6-DHT (as the creatinine sulphate, Sigma) in 4 μl saline containing 0.2 mg/ml ascorbate. Stereotaxic co-ordinates [4] were A3.4, L1.5, V−3.0. Lesions of the striatum were made with 6-OHDA, 8 μg/4 μl as above, at co-ordinates A8.0, L2.5, V0.0. The intracerebral injection procedure, involving infusion of neurotoxins at a rate of 1 μl/min via a 0.3 mm cannula, has been described in detail elsewhere [8,9].

Six groups of rats (N=5) were lesioned in the MFB, 3 groups with 6-OHDA and 3 groups with 5,6-DHT. For each neurotoxin, groups of rats were pretreated with NOM (as the hydrogen maleate, Hoechst, 15 mg/kg IP), DMI (as the hydrochloride, Ciba-Geigy, 15 mg/kg IP) or saline 45 min prior to lesion. Two groups of rats (N=6) were lesioned in the striatum with 6-OHDA after a 45 min pretreatment with NOM (15 mg/kg IP) or saline.

Animals for behavioural studies were used 8 days after lesion. They were placed in automated rotometer bowls [7], after 10 min given apomorphine (as the hydrochloride, Evans Medical, 1 mg/kg IP) and resulting rotational behaviour continuously quantified for 1 hr. Rotational data were expressed as the mean turning rates of all rotations in successive 10 min intervals over each test session.

Two weeks after the lesion rats receiving MFB lesions were stunned and killed by decapitation and extracted brains dissected into striatal and cortical samples from each hemisphere which were then frozen at −40°C prior to assay. Con-

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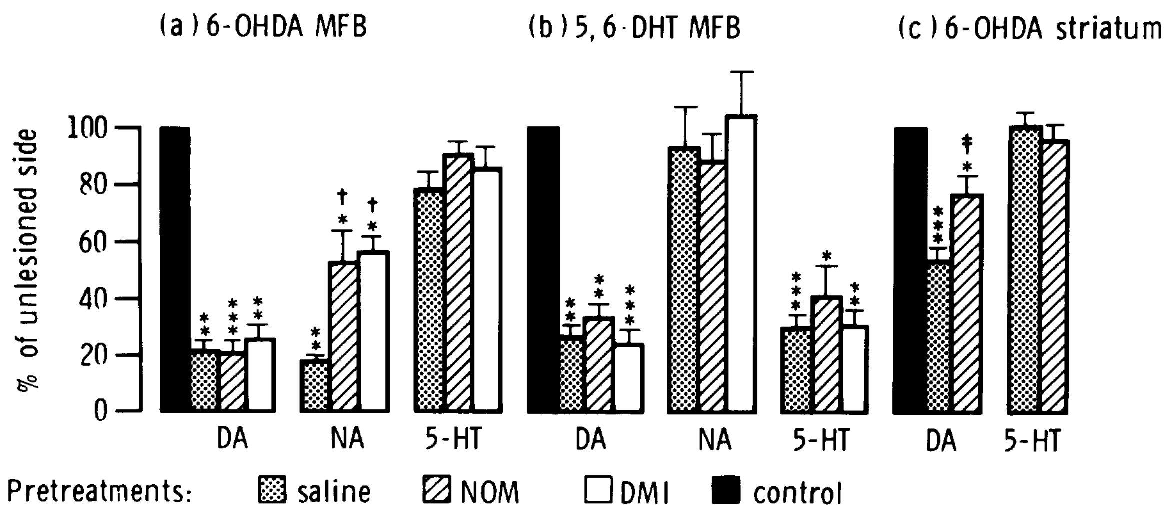

FIG. 1. Depletions of striatal DA/5-HT and cortical NA following unilateral lesions made in the MFB with (a) 6-OHDA, (b) 5,6-DHT, or in the striatum with (c) 6-OHDA. Lesions were made following a 45 min pretreatment with saline, NOM (15 mg/kg) or DMI (15 mg/kg). Results show the amine concentrations in the lesioned hemisphere expressed as a % of that in the non-lesioned hemisphere, and are the means ± SEM of 4–6 determinations. Significant depletions: *$p<0.05$; **$p<0.01$; ***$p<0.001$. Significant differences from saline pretreatment: †‡$p<0.01$.

centrations of striatal DA and 5-hydroxytryptamine (5-HT), and of cortical NA and 5-HT were determined. For rats receiving striatal lesions, only striatal tissue was taken for DA and 5-HT assay. DA and 5-HT were measured fluorimetrically and NA by a radioenzymatic technique [8,9]. Results were expressed as percentage concentration of amine in the lesioned hemisphere when compared with the non-lesioned hemisphere, and the significance of depletions determined using the dependant '*t*' test (2-tailed). Differences between groups were determined using the independent '*t*' test or the Mann-Whitney 'U' test as appropriate for the data.

RESULTS

6-OHDA injected unilaterally into the MFB substantially depleted striatal DA and cortical NA without significantly affecting 5-HT concentrations. Pretreatment with either NOM or DMI prior to lesion failed to have any effect on depletions of striatal DA induced by 6-OHDA. Both drugs, however, significantly attenuated by 50% the magnitude of the resulting cortical NA depletion, while striatal 5-HT concentrations remained unchanged (Fig. 1a).

5,6-DHT injected unilaterally into the MFB severely depleted striatal DA and 5-HT but was without effect on cortical NA concentrations. Neither NOM nor DMI had any effect on this neurochemical profile following 5,6-DHT lesions (Fig. 1b).

6-OHDA injected into the striatum depleted striatal DA without affecting 5-HT concentrations. Pretreatment with NOM significantly attenuated this DA depletion, while 5-HT concentrations remained unchanged (Fig. 1c).

Rotational responses to apomorphine in rats lesioned unilaterally in the MFB with 6-OHDA are shown in Fig. 2. Control rats and those pretreated with NOM or DMI showed indistinguishable contralateral rotation to apomorphine.

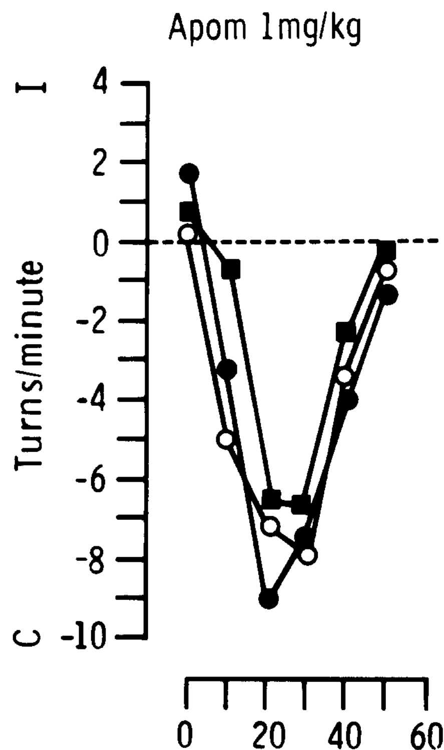

FIG. 2. Rotational responses to apomorphine (1 mg/kg IP) in animals with unilateral 6-OHDA lesions in MFB made after pretreatment with saline, NOM or DMI (see Fig. 1). Results are expressed as mean turning rate in successive 10 min intervals, in the contralateral (C) or ipsilateral (I) direction. Each curve is the mean of data from 5 animals. Control (●), NOM (○), DMI (■).

DISCUSSION

Pretreatments with NOM and DMI both attenuated the

neurotoxic action of 6-OHDA on axons of NA neurons at the level of MFB. As DMI is widely used to 'protect' NA neurons from degeneration induced by 6-OHDA [2], this action presumably reflects the ability of NOM as well as DMI to inhibit the incorporation of the neurotoxin into NA neurons via *in vivo* blockade of uptake mechanisms. Neither pretreatment influenced the neurotoxic action of 6-OHDA on the axons of DA neurons in MFB, and this was reflected functionally in indistinguishable contralateral rotational responses to apomorphine between the three MFB-lesioned groups. Similarly, neither NOM nor DMI affected the depletions of DA induced by MFB injections of 5,6-DHT, a neurotoxin widely shown to be non-specific for indoleamine neurons by inducing substantial degeneration of DA neurons [1,9]. This profile was in contrast to the action of NOM pretreatment in markedly attenuating the depletion of striatal DA induced by intrastriatal injections of 6-OHDA. This latter effect would be consistent with inhibition of striatal DA uptake processes *in vivo* by NOM. We have previously shown that identical sham injections of the saline/ascorbate vehicle for intracerebral 6-OHDA and 5,6-DHT into MFB induce only a small depletion (−20%) of striatal DA, without influencing NA and 5-HT parameters, while sham injections of saline/ascorbate vehicle made in to the striatum failed to influence striatal neurochemical parameters [8,9]. Therefore the present profile of results cannot be explained in terms of non-specific damage induced by the lesion procedure.

A corollary of this interpretation would be that NOM has less influence on axonal compared with terminal uptake processes for DA. This would imply a differential penetration of NOM into these different cerebral regions or else intrinsic differences in either the sensitivity of axonal and terminal uptake mechanisms to pharmacological inhibition or distinct structural requirements for uptake inhibition at these two sites. It cannot be excluded that substantially higher doses of NOM might have afforded protection against DA depletions induced by MFB injections of neurotoxins. While NOM is a slightly more potent inhibitor of NA than of DA reuptake processes in terminal regions (hypothalamus and striatum respectively) *in vitro* [3,5], their comparative potencies at non-terminal uptake mechanisms have not been systematically evaluated. Alternative considerations of differential sensitivity to or structural requirements for axonal and terminal uptake inhibition would imply heterogeneity of uptake processes at distinct positions on the DA neuron.

The failure of both NOM and DMI pretreatments to influence the neurotoxic action of 5,6-DHT on serotonergic neurons would be consistent with an inability to inhibit reuptake processes *in vivo*, as they fail to do *in vitro* [3,5].

As NOM and DMI pretreatments markedly attenuated depletions of forebrain NA induced by MFB injections of 6-OHDA but did not influence striatal DA depletions or contralateral rotational responses to apomorphine, these behavioural sequelae do not appear to be modulated by NA depletions that often accompany 6-OHDA lesions of DA neurons.

In summary the present results indicate NOM to attenuate the neurotoxic action of intrastriatal 6-OHDA on DA neurons, consistent with DA uptake inhibition *in vivo*, but not the action of 6-OHDA applied on to axons of DA neurons. If these distinct actions involve the differential penetration of NOM into various cerebral regions, NOM may be a useful tool only for manipulating the neurochemical and behavioural sequelae of neurotoxins applied at particular locations. If these distinct actions reflect differences in sensitivity or specificity of axonal and terminal uptake systems, heterogeneity of DA reuptake mechanisms is indicated.

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