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Dopamine and 5-HT receptor sensitivity does not correlate with neostriatal dopamine or 5-HT content

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Abstract. To explore associations of neostriatal (NST) endogenous levels of dopamine (DA) and serotonin (5-HT) with sensitivity of their receptors, graded doses of 6-hydroxydopamine HBr (0 to 400 μg, ICV; 6-OHDA; desipramine pretreatment, 20 mg/kg IP) were given to rats between birth (P 0) and P 42. Numbers of vacuous chewing movements (VCMs) induced by SKF 38393 or *m*-chlorophenylpiperazine (*m*-CPP), respective DA D_1 and 5-HT_2 agonists, were subsequently determined. Enhanced SKF 38393-induced VCMs occurred when NST DA was reduced 97%-98% by high dose 6-OHDA (100-134 μg) at P 0 or P 3, but not in rats with 95%-97% loss in DA produced by 6-OHDA at P7 (134 μg) or P3 (67 μg). Enhanced *m*-CPP-induced VCMs occurred even when NST 5-HT content was not elevated after 6-OHDA (134 μg at P 10). Accordingly, D_1 and 5-HT receptor sensitivity is not correlated with respective NST DA and 5-HT contents. The stage of ontogeny at the time of DA denervation may be the governing influence on receptor sensitivity.

Key words: dopamine, dopamine receptors, neostriatum, oral dyskinesias, receptor supersensitivity, serotonin, 5-HT receptors, 6-hydroxydopamine

INTRODUCTION

Breese and co-workers first demonstrated that dopamine receptor supersensitivity developed in rats in which dopamine (DA) neurones were largely destroyed in the brain of newborn rats (Breese et al. 1984). This was reflected as an enhanced stereotypic or locomotor response to DA agonists, primarily those acting at DA D_1 receptors (Breese et al. 1985a,b, 1987). However, in testing with a selective DA D_1 receptor agonist like SKF 38393 [(±)-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol], it was found that DA receptor supersensitivity developed gradually with repeated SKF 38393, a process that has been termed 'priming' of DA D_1 receptors (Criswell et al. 1989).

Also in the mid 1980s Rosengarten and associates found that purposeless oral activity or chewing movements, sometimes called vacuous chewing (Waddington 1990), was induced in rats by DA receptor agonists, primarily those acting at DA D_1 receptors (Rosengarten et al. 1983, 1986). Later, Stewart et al. (1989) found that serotonin (5-HT) agonists would also induce this behaviour in rats.

In a combined attempt to study the phenomenon known as DA receptor supersensitivity and to explore new animal models of tardive dyskinesia, the effects of DA and 5-HT receptor agonists on oral activity was determined in rats in which DA neurones were largely destroyed near the time of birth by administering the catecholamine neurotoxin, 6-hydroxydopamine (6-OHDA). The following report summarizes some of the important findings of these studies.

METHODS

Animal treatment

Timed-pregnant Sprague Dawley albino rats were treated at birth or at intervals up to 42 days after birth with desipramine hydrochloride (20 mg/kg IP, base form) 1 h before bilateral intracerebroventricular (ICV) injections of 6-OHDA hydrobromide (10 to 400 μg, base form, on each side). Control rats received the same dose of desipramine, but in combination with the vehicle, saline (0.85%) containing ascorbic acid (0.1%), in place 6-OHDA. After weaning at 28 days, rats were housed by sex and studied when at least 6 weeks old.

Animal testing

To observe oral activity, rats were placed in single clear plastic cages (48 x 26 x 18 cm or 48 x 26 x 36 cm, depending on the size of the rat) in a quiet, well-ventilated and well-lighted room. In some cases there was a steel grid floor. Cages of the same height were used for any single test session.

After an accommodation of at least 1 h, rats were treated with vehicle, SKF 38393 HCl or *m*-chlorophenylpiperazine 2HCl (*m*-CPP 2HCl), respective DA D_1 or 5-HT_{2C} receptor agonists. Each rat was observed one at a time, for 1 min every 10 min, over a 1 h period, starting 10 min after the indicated vehicle or agonist treatment. Numbers of vacuous chewing movements were counted. Vacuous chewing represents spontaneous chewing, which is not directed onto any physical matter and is not associated with eating.

In some instances, the respective DA D_1, 5-$HT_{2A,2C}$, or 5-HT_{2A} receptor antagonists, SCH 23390 [(R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; 0.30 mg/kg IP), mianserin HCl (1.0 mg/kg IP) or ketanserin tartrate (5.0 mg/kg) were administered 1 h before observation.

Neurochemical analysis

At the conclusion of the study rats were decapitated and the neostriatum was removed from surrounding brain matter. These were frozen on dry ice, then stored frozen at -80° C until the time of monoamine extraction. To accomplish this, single neostriata were sonicated in 0.10 M trichloroacetic acid containing 0.20 mg/ml of cysteine and the internal standard, 0.20 nmol/ml of 5-hydroxyindolecarboxylic acid.

Neostriatal concentrations of DA and 5-HT were assessed, using a Bioanalytical Systems liquid

chromatograph with an Econosphere C18 analytical column (5 µm, 4.6 x 150 mm) having a mobile phase of 0.10 M monochloroacetic acid, 1mM EDTA, 220 mg/l of sodium octanesulfonic acid and 8% acetonitrile with a pH of 2.6 at a flow rate of 1.3 ml/min and temperature of 40° C. A glassy carbon electrode was used at a potential of +0.75 V (see Gong et al. 1992).

DA receptor binding parameters

The neostriatum was assessed for DA D_1 receptor binding parameters in the following way. After teflon on glass homogenization in 100 volumes of 50 mM Tris buffer (pH 7.4), and 2 centrifugation steps at 35,100 x g for 10 min each time at 4° C, tissue pellets were resuspended in Tris buffer containing 120 mM NaCl, 5 mM KCl and 2 mM $CaCl_2$. Aliquots were added to [^{3}H]SCH 23390 (50-2500 pM) and incubated for 40 min at 37° C in a shaking water bath. Incubates were then rapidly filtered under partial vacuum on Whatman GF/F glass fibre filters. After washing 3 times with ice-cold Tris salt solution, filters were dried, then added to fluor for determination of tritium activity. A GraphPAD program (ISI software, Philadelphia, PA) was used to calculate the B_{max} and K_d values for each sample (Hamdi and Kostrzewa 1991).

Data analysis

Behavioural and biochemical data of treated and control groups were compared by an analysis of variance (ANOVA), followed by the post-ANOVA test of Newman-Keuls.

RESULTS

Oral activity responses to SKF 38393 and *m*-CPP in intact and 6-OHDA-lesioned rats

In rats lesioned with a high dose of 6-OHDA (134 µg ICV) at 3 days after birth, the oral activity responses to SKF 38393 and *m*-CPP were greatly enhanced. Moreover, this was observed after the first dose of SKF 38393 or *m*-CPP in lesioned rats, indicating that a priming process was not required for sensitization of the DA D_1 and 5-HT_{2C} receptors associated with this behaviour. In other words, there is overt supersensitization of these receptors for oral activity behaviour, since a priming process is not needed in order for enhanced behavioural processes to be observed.

In these 6-OHDA-lesioned rats (134 µg 6-OHDA ICV; desipramine pretreatment) SKF 38393 HCl induced a graded increase in oral activity in the dose range 0.03 to 3.0 mg/kg, with the peak effect of 30 chewing sequences occurring at the 0.3 mg/kg dose (Kostrzewa and Gong 1991). With this dose of SKF 38393 there is little or no induction of stereotyped and locomotor activities (Gong et al. 1992). In intact rats SKF 38393 at this dose level had virtually no effect on these behaviours and did not induce oral activity (Kostrzewa and Gong 1991, Gong et al. 1992). The DA D_1 receptor antagonist, SCH 23390 (0.30 mg/kg, IP, 1 h) attenuated the response to SKF 38393 in lesioned rats.

The 5-HT_{2C} receptor agonist, *m*-CPP, produced a bell-shaped dose-effect curve, in the range 0.3 to 6.0 mg/kg, in intact and 6-OHDA-lesioned rats (134 µg 6-OHDA ICV; desipramine pretreatment). The peak effect, 60 chewing sequences in 6-OHDA-lesioned rats or 15 chewing sequences in intact rats, occurred at the dose of 3.0 mg/kg. The 6.0 mg/kg dose of *m*-CPP 2HCl was associated with less vacuous chewing. The 5-$HT_{2A,2C}$ receptor antagonist, mianserin (1.0 mg/kg, IP, 1 h) attenuated the response to *m*-CPP in intact and lesioned rats while the 5-HT_{2A} receptor antagonist, ketanserin (5.0 mg/kg, IP, 1 h) did not (Gong et al. 1992, Gong and Kostrzewa 1992).

Effects of 6-OHDA treatment on neostriatal DA and 5-HT contents

DOSE-RELATED EFFECTS OF 6-OHDA ON DA AND 5-HT CONTENTS

6-OHDA, administered ICV to rats at 3 days after birth (desipramine pretreatment), effectively

altered the development of neostriatal levels of DA in rats that were assessed in adulthood. This effect occurred throughout the entire dose range of 6-OHDA, even with the lowest 6-OHDA dose (10 μg). As illustrated in Fig. 1 and Table I, low doses of 6-OHDA (10 and 20 μg) had a modest influence of DA content of the neostriatum. The 40 μg dose of 6-OHDA reduced neostriatal DA by about 90%, while higher 6-OHDA doses produced a 97-98% reduction in neostriatal DA content.

Neostriatal 5-HT content, which appears to be reflective of the relative density of innervation of neostriatum by 5-HT fibres, is not as severely influenced by 6-OHDA. Modest doses of 6-OHDA (10 or 20 μg) had no effect on neostriatal 5-HT content. However, at doses of 6-OHDA that altered neostriatal DA content by 90% or more, 5-HT content became altered. A 2-fold elevation in 5-HT content, or more, is observed in the neostriatum of adult rats that received high doses of 6-OHDA (ICV) at 3 days after birth (Gong et al. 1993). The relationship between DA depletion and 5-HT elevation in the neostriatum in such rats is illustrated in Fig. 1.

AGE-RELATED EFFECTS OF 6-OHDA ON DA AND 5-HT CONTENTS

The effect of 6-OHDA on neostriatal DA innervation, as reflected by neostriatal DA content, is dependent on the age of rats at the time of 6-OHDA treatment. This ontogenetic effect is indicated in Table I and illustrated in Fig. 2. In summary, when administered to rats up to 10 days after birth, at a dose of 134 μg or higher, 6-OHDA prevented the development of neostriatal DA by 90%. These marked reductions in DA content were associated with an elevation in neostriatal 5-HT content, with the greatest change occurring in rats that were treated at birth or 3 days from birth. When 6-OHDA was administered at 7 days from birth, a subsequent 95% reduction in neostriatal DA was associated with a modest increase in neostriatal 5-HT content (28%) (Table I). When 6-OHDA was administered at 10 days from birth, the 95% reduction in neostriatal DA was not associated with an elevation in 5-HT content. At later stages of ontogeny, the same or higher dose of 6-OHDA had less of an effect on

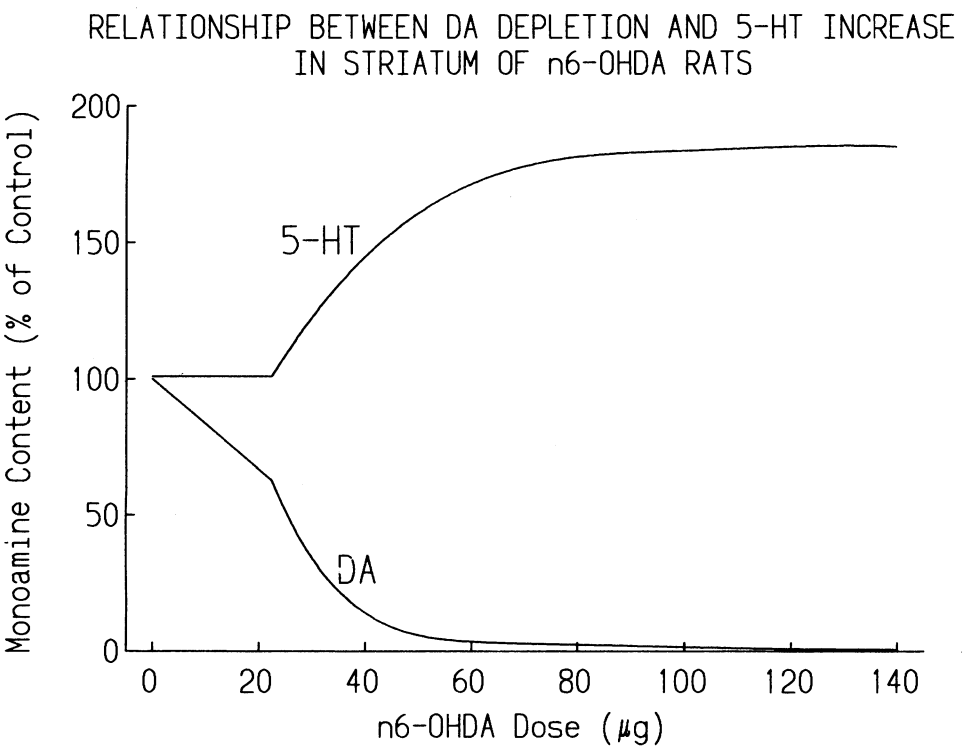

Fig. 1. Dose effects of 6-OHDA, (desipramine pretreatment, 20 mg/kg IP, 1 h; 3 days after birth) on DA and 5-HT contents of neostriatum, assessed in adulthood. Each value represents the mean and SEM of 5 or more rats. * $P<0.05$, ** $P<0.001$ vs. vehicle control group (0 on x-axis).

TABLE I

Effects of bilateral intracerebroventricular (ICV) 6-OHDA treatments (desipramine pretreatment, 20 mg/kg IP, 1 h) on DA and 5-HT contents of rat neostriatum, when analysed in adulthood; and on DA and 5-HT receptor supersensitivity (DARSS, 5-HTRSS), when assessed in adulthood

	ICV Treatment		% Control		DARSS	5-HTRSS
			DA	5-HT		
P0	6-OHDA, 134 µg		2	151	YES	YES
P3	6-OHDA, 134 µg		2	182	YES	YES
		100 µg	2	197	YES	YES
		67 µg	3	150	NO	YES
		40 µg	12	144	NO	NO
		20 µg	63	(112)	NO	NO
		10 µg	86	(94)	NO	NO
P7	6-OHDA, 134 µg		5	128	NO	YES

( ) indicates changes that are not significantly different from vehicle treated controls. All other values are different from control, $P<0.05$ or less.

neostriatal DA content, while 5-HT content was not altered (Kostrzewa et al. 1993).

Association of neostriatal DA and 5-HT contents with the induction of oral activity by DA and 5-HT agonists

When administered to rats at birth or 3 days after birth, ICV doses of 6-OHDA of 100 or 134 µg effectively produced a 98% life-long reduction in neostriatal DA content (Table I). Associated with this changes was the life-long 50 to 100% increase in neostriatal 5-HT content. In each instance the respective DA D_1 and 5-HT_{2C} receptor agonists, SKF 38393 and *m*-CPP, produced enhanced oral activity responses vs. that observed with identical doses in intact rats (Table I). The 67 µg dose of 6-OHDA, administered to rats at 3 days after birth, was associated with the noted alterations in neostriatal DA and 5-HT contents. In these rats, however, an enhanced oral activity response of *m*-CPP was still observed while that of SKF 38393 was not (Gong et al. 1993). A lower dose of 6-OHDA, 40 µg, administered to rats at 3 days after birth, was also associated with an

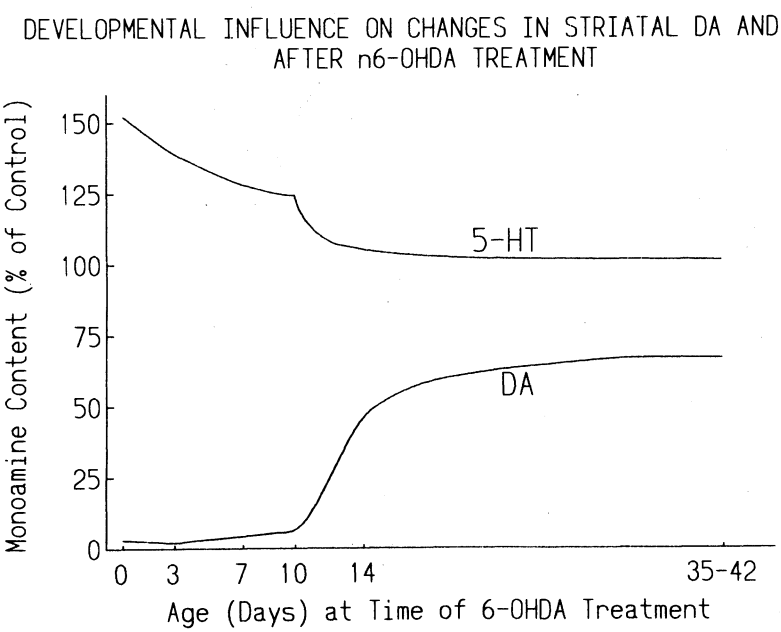

Fig. 2. Long-lived alterations in endogenous DA and 5-HT contents of the neostriatum of rats treated at different ages with 6-OHDA (desipramine pretreatment, 20 mg/kg IP, 1 h). In this study 6-OHDA was administered as 134 µg base form ICV, except that 200 µg x 2 (1 week interval) was administered to rats at 35 days of age. Rats were at least 3 months old at the time of monoamine analysis (see Kostrzewa et al. 1993).

approximate 50% elevation in neostriatal 5-HT content, but in these rats an enhanced oral activity response to *m*-CPP was not observed. Therefore, it appears that DA receptor supersensitivity (DARSS) and 5-HTRSS are lost even when changes in neostriatal DA and 5-HT contents are similar or identical to that observed with higher 6-OHDA doses in rats of the same age at the time of treatment. Accordingly, neostriatal DA and 5-HT contents do not seem to be major determinants for the phenomena of DARSS and 5-HTRSS.

Using a similar experimental approach with rats at different stages of postnatal ontogeny, the constant 134 μg dose of 6-OHDA was associated with at least a 95% life-long reduction in neostriatal DA and at least a 25% life-long elevation in neostriatal 5-HT content, when administered to rats either at birth, 3 days after birth, or 7 days after birth (Table I). Despite this, enhanced oral activity responses to SKF 38393 were absent in rats treated with 6-OHDA at 7 days after birth. Enhanced oral activity responses to *m*-CPP were observed in rats treated at 10 days after birth with 6-OHDA, even though there was no alteration in neostriatal 5-HT content in adulthood. However, in rats treated with 6-OHDA at 14 days after birth or later, enhanced oral activity responses to *m*-CPP were not observed in adulthood. This series of studies indicates that there is no clear association between (1) DA or 5-HT agonist-induced behaviours and (2) long-lived alterations in neostriatal DA and 5-HT contents (Table I) (Kostrzewa et al. 1993).

Effect of neonatal 6-OHDA treatment on DA D_1 and D_2 receptor affinity and number

In adult rats that were lesioned neonatally with 6-OHDA (134 μg at 3 days after birth), there was no change in the B_{max} or K_d for DA D_1 or D_2 receptors in the neostriatum, when [^{3}H]SCH 23390 or [^{3}H]spiperone were used as the respective ligands.

DISCUSSION

This series of studies demonstrates that there is an enhancement of DA D_1 and 5-HT agonist-induced oral activity in rats there were lesioned early in postnatal ontogeny with 6-OHDA and that this effect is not accompanied by a change in the B_{max} or K_d for DA D_1 receptors. Lacking a suitable ligand, an assessment of 5-HT_{2C} receptor binding parameters has not been possible.

The major finding is that the behavioural supersensitization of DA D_1 and 5-HT_{2C} receptors is not closely correlated with a change in neostriatal content of DA or 5-HT. The neostriatum represents a focus for DA and 5-HT agonist induction of oral activity in rats (Plech et al. 1995). It is apparent from Table I that DARSS is present after a 100 μg, but not after a 67 μg ICV dose of 6-OHDA, administered to rats 3 days after birth. In each instance, neostriatal content was reduced by 97 or 98%. Similarly, a 134 μg dose of 6-OHDA, administered to rats at 7 days after birth, reduced neostriatal DA content by 95%, but was not associated with DARSS. On the basis of these findings it appears that a large reduction in neostriatal DA content may be necessary for DA D_1 receptor supersensitization, but there is no good correlation of neostriatal DA content with DARSS.

Enhanced oral activity responses to *m*-CPP, a 5-HT_2 receptor agonist, are present in rats treated at 3 days after birth with a 67 μg dose of 6-OHDA, but not after a 40 μg dose, despite the fact that each treatment was accompanied by a 44 to 50% elevation in neostriatal 5-HT content. A 134 μg dose of 6-OHDA, administered to rats at 10 days after birth, was associated with 5-HTRSS but not a change in neostriatal content of 5-HT. These findings clearly demonstrate that 5-HTRSS is not associated with changes in neostriatal 5-HT content.

The reduction in DA content and elevation in 5-HT content in the neostriatum of rats treated neonatally with 6-OHDA is reflective of the loss of DA fibre innervation and presence of 5-HT fibre hyperinnervation, respectively. It is logical to assume that such alterations can influence the number or sensitivity of DA and 5-HT receptors. It is well-known that a change in DA D_1 receptor sensitivity is not correlated with the number of DA D_1 receptors in neostriatum (Breese et al. 1985a,b, Kostrzewa 1995).

Therefore, the focus for the described studies was on the possible association between neostriatal monoamine content and enhanced behavioural responses to agonists. The lack of a correlation may be related simply to the fact that there are other brain sites that may be as crucial or even more critical for DA and 5-HT agonist-induced oral activity in rats. However, it must be recognized that the neostriatum is not an anatomically homogenous structure, but rather, has a somatotopic organization with striosome and matrix compartments (Gerfen et al. 1988). Moreover, each of these compartments has different populations of DA D_1 and D_2 receptors. Future studies into the association between receptor sensitivities and monoamine content may be more appropriately focused on this organizational feature.

The stage of ontogeny at the time of DA fibre destruction by 6-OHDA is also a major consideration relating to sensitization of DA and 5-HT receptors. Early in ontogeny DA fibres are likely to exert an influence on the development and maturation of other neurones that arise within or traverse portions of the neostriatum or other forebrain structures. The 5-HT fibre proliferation that occurs subsequent to DA fibre destruction could represent another element whereby different neurochemical systems compete with one another for innervation of target cells. In essence, the destructive loss of DA neurones after 6-OHDA treatment during postnatal ontogeny, may cause major reordering of neuronal systems. This may be the most important determinant of DARSS and 5-HTRSS.

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