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Differentiation of the dopamine mechanisms mediating stereotyped behaviour and hyperactivity in the nucleus accumbens and caudate-putamen

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A number of dopamine agonists were applied intracerebrally to the nucleus accumbens and caudate-putamen of rat in an attempt to differentiate the dopamine mechanisms in these nuclei which mediate hyperactivity and stereotyped behaviour. The major effect of dopamine was to induce hyperactivity from the nucleus accumbens and stereotypy from the caudate-putamen; *N*-n-propyl-norapomorphine induced hyperactivity and stereotypy from the nucleus accumbens whilst apomorphine induced a marked stereotypy from the caudate-putamen, modest stereotypy from the nucleus accumbens and no hyperactivity. In contrast to apomorphine, 2-(*NN*-dipropyl)amino-5,6-dihydroxy TN‡ induced a more marked stereotypy from the nucleus accumbens and, again, no hyperactivity. The major effect of 2-(*NN*-diethyl)amino-5,6-dihydroxy TN was to cause an intense hyperactivity from the nucleus accumbens and marked stereotypy from the caudate-putamen whilst the primary amine, 2-amino-5,6-dihydroxy TN induced hyperactivity and stereotypy from both areas. The marked hyperactivity and stereotyped responses were inhibited by haloperidol, but not by α- or β-blockers. These data would indicate that there may be different dopamine mechanisms in the nucleus accumbens and caudate-putamen for the mediation of hyperactivity and stereotyped behaviour.

It is now known that both striatal and mesolimbic brain regions play important roles in the modulation of dopamine-dependent stereotyped behaviour and hyperactivity (Kelly, Seviour & Iversen, 1975; Costall, Marsden & others, 1977), and recent work would suggest that the dopamine mechanisms within the two regions may be different. Thus, apomorphine causes stereotyped biting when injected into the caudate-putamen but has an inconsistent effect when injected into the nucleus accumbens (Costall, Naylor & Neumeyer, 1975a, b), and *N*-n-propyl-norapomorphine (NPA) induces both stereotypy and hyperactivity from the nucleus accumbens but has only an irregular and very weak activity in the caudate-putamen (Costall & others, 1975a, b). More recently, we have been investigating the abilities of a number of 2-amino TN‡ derivatives to cause these same behavioural changes on intracerebral injection and, again, we have observed differences in the responses following injections into the caudate-putamen and nucleus accumbens (Cannon, Lee & others, 1977; Costall, Naylor & others, 1977). We now report data which indicate *differences* in the dopamine mechanisms mediating stereotypy and hyperactivity *within* the extrapyramidal caudate-putamen and mesolimbic nucleus accumbens and, more speculatively, *between* these areas. We selected apomorphine, NPA, 2-amino-5,6-dihydroxy 1,2,3,4 tetrahydronaphthalene (5,6-diOHATN), 2-(*NN*-diethyl)amino-5,6-dihydroxy 1,2,3,4 tetrahydronaphthalene (*NN*-diEt-5,6-diOHATN), 2-(*NN*-dipropyl)amino-5,6-dihydroxy 1,2,3,4 tetrahydronaphthalene (*NN*-diPr-5,6-diOHATN) and dopamine itself for use in these studies, and established the dopamine dependency of the observed responses by assessing the antagonistic abilities of propranolol, aceperone and haloperidol.

MATERIALS AND METHODS

Male, Sprague-Dawley (CFE) rats, 250–300 g, were prepared for intracerebral drug administration by stereotaxically implanting bilateral guide cannulae constructed from 0·65 mm diameter stainless-steel tubing fixed in Perspex blocks 6 mm apart for intrastriatal injections and 3·2 mm apart for injections into the nucleus accumbens (see Costall & others, 1975a, for details). The tips of the guides were located at Ant 8·0, Vert +3·0, Lat ±3·0 (caudate-putamen) and Ant 9·0, Vert +2·5, Lat ±1·6 (nucleus accumbens) (De Groot, 1959). Stainless-steel stylets kept the guides patent until animals were used 10–14

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‡ TN ≡ 1,2,3,4 tetrahydronaphthalene, ATN ≡ 2-amino TN.

days after surgery. Rats were manually restrained during the intracerebral injection procedure when the stylets were replaced by injection units (stainless-steel, 0·3 mm diameter) which extended 1·5 mm or 2·5 mm below the guides (caudate-putamen and nucleus accumbens respectively) and so terminated at the centre of these nuclei (Vert +1·5 for the caudate-putamen, Vert 0 for the nucleus accumbens). The injection units were coupled to Agla micrometer syringes which were used to deliver 1 or 2 μl (nucleus accumbens and caudate-putamen respectively) drug or solvent solution simultaneously to both hemispheres. Solutions were delivered within 5 s and a further 55 s were allowed for deposition of drug. Animals were used on one occasion only and were then killed for histological examination of the cannulae locations. The brain of every tenth rat was examined: all locations were found to be correct for injections into the caudate-putamen or nucleus accumbens, and were indistinguishable from those previously reported (see Costall & others, 1975a).

Hyperactivity was measured in Perspex cages (30 cm × 20 cm and 15 cm high) fitted with photocells. One rat was placed in each cage and the number of light beam interruptions caused by that rat within each 5 min period was recorded for at least 7 h. The activity of 30 rats was assessed on any one occasion. During each 5 min period animals were also assessed by experienced observers for the presence or absence of locomotor activity and any stereotyped behaviour was recorded. Any count obtained which was not validified by observation of locomotor activity was excluded (termed non-responders in Figs 1 and 2). These counts were due to the recording of stereotyped movements which invariably occurred when a stereotyped animal's position was coincident to the light beam. Stereotypy was assessed on a simple scoring sytem where 0 = no stereotypy, 1 = periodic sniffing and/or repetitive head and limb movements, 2 = continuous sniffing and/or repetitive head and limb movements, 3 = periodic gnawing, biting or licking, 4 = continuous gnawing, biting or licking. When using this sytem the presence of locomotor activity does not exclude the concomitant development of stereotypy. If an animal was rated as active and stereotypy was scored 0, then the count recorded was taken as a reliable index of locomotor activity. If, however, an animal was rated as active but at the same time was exhibiting some stereotyped movements this may have increased or decreased the locomotor count, and may or may not have significantly contributed to the locomotor count. However, it must be emphasized that these interactions do not invalidate the observations, an animal was either hyperactive and/or stereotyped, and it must be appreciated that for those drugs which at certain doses only caused a combined behaviour, the count may not be a *precise* expression of *locomotor* activity, although this lack of precision can in no way alter the fundamental interpretation of the results.

For both the stereotypy and hyperactivity tests animals received nialamide (100 mg kg^{-1}, i.p.) 2 h before an intracerebral injection. Control rats receiving intracerebral solvent injections were run concurrently with the experimental animals. The bilateral injection of solvent into the nucleus accumbens generally caused an immediate hyperactivity (5–10 counts/5 min) of maximum duration 6 min. Solvent injections into the nucleus accumbens never induced stereotyped behaviour as a component of the injection artifact. Bilateral injections of solvent into the caudate-putamen generally caused an immediate but brief (2–4 min) period of hyperactivity (< 10 counts/5 min) occasionally associated with biting. Some rats failed to give these characteristic injection artifacts but remained completely quiet after intracerebral solvent injections.

Experiments were carried out in a sound-proofed, diffusely illuminated room maintained at 21 ± 1°. The distribution of values obtained for both stereotypy and hyperactivity were sufficiently close to normal to allow the application of parametric statistics. Differences between responses were analysed using the Students '*t*'-test.

Drugs for intracerebral injection were prepared in nitrogen bubbled distilled water. The 2-aminoTN-derivatives were synthesized as the HBr. Other drugs used were dopamine, HCl (Koch-Light), apomorphine, HCl (Macfarlan Smith), (−)NPA HCl (Neumeyer). All peripherally administered drugs were given by the intraperitoneal route in a volume of 1 ml kg^{-1} with doses calculated as the base: haloperidol (Janssen) was prepared in 1% lactic acid, aceperone (Janssen) in the minimum quantity of *NN*-dimethylformamide made up to volume with distilled water, propranolol HCl (ICI) in distilled water and nialamide (Sigma) in a minimum quantity of HCl made up to volume with distilled water.

RESULTS

Dopamine was shown to induce a dose-dependent hyperactivity when injected into the caudate-putamen or nucleus accumbens. Both effects developed within 2 h and persisted for at least 5 h. The hyperactivity induced from the nucleus accumbens was of greater

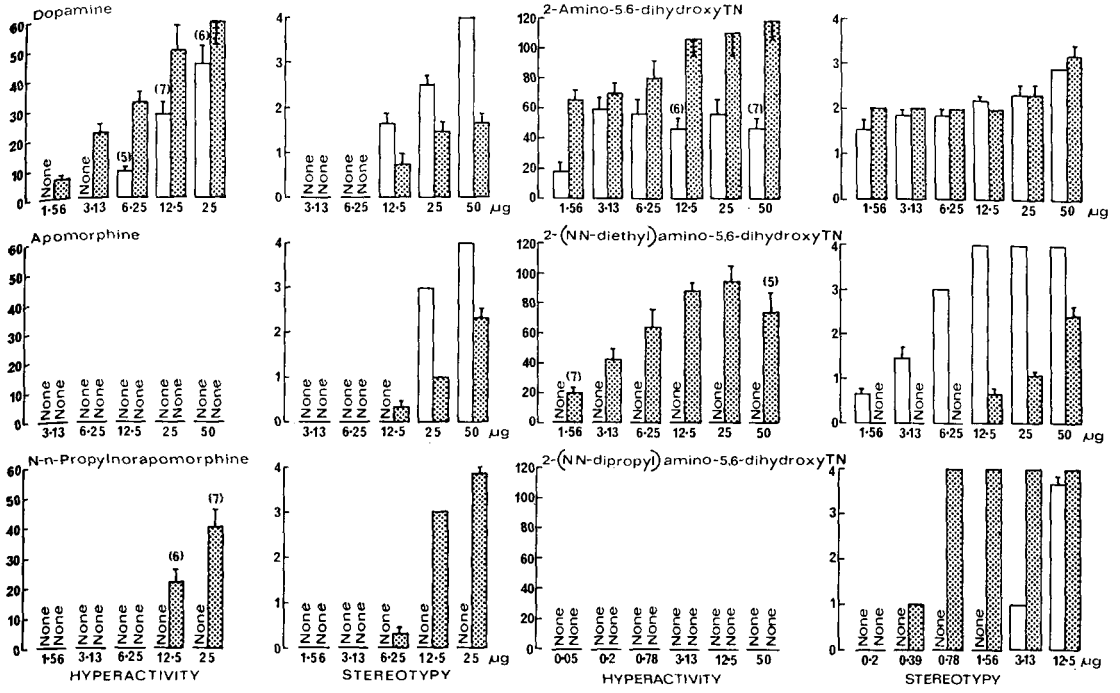

FIG. 1

FIG. 2

FIG. 1. Hyperactivity and stereotyped behaviour induced by the intracerebral administration of dopamine, apomorphine and *N*-n-propylnorapomorphine into the caudate-putamen (open columns) and nucleus accumbens (stipuled columns). Doses are indicated in μg administered bilaterally 2 h after pretreatment with nialamide (100 mg kg^{-1}, i.p.). Hyperactivity is expressed in counts per 5 min and stereotypy is scored (see Methods). 8 rats were used at each dose level of drug and the mean maximal response is shown. When less than 8 animals gave a positive response, non-responders (as defined on p. 338) were excluded and the number of rats contributing to the mean value is indicated in parentheses. Standard errors of the means are indicated.

FIG. 2. Hyperactivity and stereotyped behaviour induced by the intracerebral administration of 5,6-diOHATNs*NN*-diEt-5,6-diOHATN and *NN*-diPr-5,6-diOHATN into the caudate-putamen (open columns) and nucleus accumbens (stipuled columns). Doses are indicated in μg administered bilaterally 2 h after pretreatment with nialamide (100 mg kg^{-1}, i.p.). Hyperactivity is expressed in counts per 5 min and stereotypy is scored (see Methods). 8 rats were used at each dose level of drug and the mean maximal response is shown. When less than 8 animals gave a positive response, non-responders (as defined on p. 338) were excluded and the number of rats contributing to the mean value is indicated in parentheses. Standard errors of the means are indicated.

intensity and was associated only with a weak stereotyped sniffing. However, dopamine injected into the caudate-putamen induced intense biting which achieved maximum intensity at 50 μg: at this dose the restricted biting movements were associated with a reduced hyperactivity response (Fig. 1).

In contrast to dopamine, apomorphine completely failed to enhance locomotor activity when injected directly into the caudate-putamen or nucleus accumbens in a wide dose range of 3·13–50 μg bilateral. Apomorphine did, however, induce a biting response from the caudate-putamen which was of equal intensity to that induced by dopamine (Fig. 1) although the onset was more rapid (4–9 min) and the duration greatly reduced (20–40 min). Again, sniffing and repetitive head and limb movements were the major components of the stereotypy induced by apomorphine injected into the nucleus accumbens (Fig. 1). In contrast to both dopamine and apomorphine, NPA failed to induce either hyperactivity or stereotyped behaviour when injected into the caudate-putamen, although both behavioural responses were observed when NPA was injected into the nucleus accumbens (Fig. 1). At 25 μg NPA both hyperactivity and stereotyped biting were apparent—animals were seen to be continuously moving about the activity cages with their mouths full of shavings. Close observation of this behaviour on videotape emphasized the stereotyped nature of the oral chewing movements of these rats even

though their movements were not restricted. The effect of NPA in the nucleus accumbens developed within 7–14 min and lasted for at least 4 h.

The stereotypic effects of 5,6-diOHATN in the caudate-putamen and nucleus accumbens were indistinguishable: the responses were characterized by repetitive sniffing and head movements and a very periodic biting only developed at 50 μg (Fig. 2). It also induced hyperactivity from both brain regions although the intensity of the response was significantly greater ($P < 0{\cdot}001$) when injections were made into the nucleus accumbens. (Fig. 2) The effects of 5,6-diOHATN in both the caudate-putamen and nuclus accumbens developed within approximately 90 min and lasted for at least 6 h.

The activity spectrum of *NN*-diEt-5,6-diOHATN contrasted with that of all other agents so far discussed. It caused intense stereotyped biting when injected into the caudate-putamen whilst only a very weak sniffing response developed after injections into the nucleus accumbens but, in complete contrast, this drug failed to induce any hyperactivity from the caudate-putamen whilst locomotor activity was significantly increased ($P < 0{\cdot}001$) when injections were made into the nucleus accumbens (Fig. 2). The time courses of these effects were similar to those described for the primary amine, excepting that the onset of action tended to be more rapid and generally occurred within 60 min of injection.

NN-dipropyl substitution of the primary amine further changed the spectrum of activity since *NN*-diPr-5,6-diOHATN was void of ability to enhance locomotor activity either when injected into the caudate-putamen or into the nucleus accumbens but, in contrast to the *NN*-diethyl derivative, it was far more active as a stereotypic agent in the nucleus accumbens; significantly larger doses ($P < 0{\cdot}001$ when comparing threshold doses) were required to induce biting from the caudate-putamen (Fig. 2). The effects of *NN*-diPr-5,6-diOHATN were apparent within 30 min of its injection and persisted for at least 6 h.

The stereotyped biting responses induced by dopamine (50 μg), apomorphine (50 μg), 5,6-diOHATN (3·13 μg) and *NN*-diEt-5,6-diOHATN (12·5 μg) when they were injected into the caudate-putamen, and by 5,6-diOHATN (3·13 μg) and *NN*-diPr-5,6-diOHATN (50 μg) injected into the nucleus accumbens, were abolished by haloperidol (0·2–0·8 mg kg^{-1}, i.p.). For each agent, the blocking effects of haloperidol became apparent within 10 min and persisted throughout the duration of drug action (apomorphine) or for at least 5 h. The stereotyped biting induced by NPA was also abolished by haloperidol (0·4–0·8 mg kg^{-1}, i.p.) but, although the onset of inhibition was within 15 min, the period of complete antagonism was apparent for only 40–70 min. The same comment applies to the hyperactivity induced by NPA in the nucleus accumbens—the onset of haloperidol antagonism was within 35 min but the duration of effect was generally less than 60 min. But, the hyperactivity induced by 5,6-diOHATN and *NN*-diEt-5,6-diOHATN was inhibited by haloperidol (0·4 mg kg^{-1}, i.p.) on the time course described for biting. Similar doses abolished the hyperactivity induced by dopamine and 5,6-diOHATN in the caudate-putamen. The hyperactivity induced by dopamine in the nucleus accumbens was slightly more sensitive to haloperidol blockade, and 0·2 mg kg^{-1} abolished the effect. Again, onset was within 15 min and the inhibition persisted throughout the duration of the test. None of these behavioural effects inhibited by haloperidol were reduced by an α- or β-adrenoceptor blocking agent: aceperone and propranolol (10 mg kg^{-1}, i.p.) were totally ineffective.

DISCUSSION

In an interpretation of the present results it is important to gain some indication that the motor effects induced by the dopamine agonists are dopamine-dependent, and this was achieved using haloperidol which antagonized both the hyperactivity and stereotypy, and by using α- and β-antagonists which failed to block these responses. Secondly, it is implicit that the motor effects which are observed following the injection of dopamine agonists into discrete brain areas are due to an action within the areas of injection. It is, however, realized that some of the injected agent will diffuse through cerebral tissue and/or the ventricular system and some may be re-distributed via the vasculature to other areas of the brain. But if these were major factors to be considered then 'limitless diffusion' would preclude the results obtained in the present and many other studies. It is more difficult to interpret a lack of response after intracerebral injection which could indicate, firstly, a lack of appropriate receptors to mediate a response, secondly an action on other neurotransmitter mechanisms present within the area of injection and whose activation may oppose drug action on another system mediating the primary response, thirdly a rapid diffusion to the vasculature and subsequent inactivation and, finally, a rapid local metabolism

possibly absent in other brain areas. The first two possibilities appear the more probable but, in the absence of precise data as to the effects of most of the various agents on 5-hydroxytryptamine, noradrenaline, γ-aminobutyric acid and other neurotransmitter or modulatory substances, an interpretation of the present data is more realistically considered in terms of differences in dopamine mechanisms (this does *not* exclude a drug/dopamine interaction with the above neurotransmitters) than in dopamine receptors.

The effects observed with dopamine itself (a characteristic hyperactivity induced from the nucleus accumbens, biting from the striatum) supports the general hypothesis that mesolimbic systems are primarily concerned with locomotor activity whilst striatal mechanisms are more important for modulation of stereotypy (Pijnenburg & van Rossum, 1973; Kelly & others, 1975; Pijnenburg, Honig & van Rossum, 1975; Costall, Marsden, & others, 1977). However, the injection of dopamine into the nucleus accumbens also evoked weak stereotyped head and limb movements when using larger doses and definite locomotor activity was recorded for many animals following injection into the striatum. A 'slight to moderate increase of motor activity' following intrastriatal dopamine has also been found by Malec (unpublished data, see Pijnenburg & van Rossum, 1973) although this response is frequently dismissed as a weak effect by other workers (Fuxe & Ungerstedt, 1970; Benkert & Köhler, 1972) in the absence of quantitative data to support this claim. Differences between these results and those reported in the present study are probably related to the differences in the concentrations and volumes of dopamine used. Thus, when using small volumes, of 1 μl or less, we record, as do other workers, an almost exclusive stereotyped response (Costall, Naylor & Pinder, 1974). However, when using a 2 μl volume for injection we record a hyperactivity in many animals which is followed by the development of stereotyped biting at the higher doses. Thus the results would indicate that both the nucleus accumbens and caudate-putamen have a potential to mediate both locomotor activity and stereotypy and this is emphasized by the results using 5,6-diOHATN which was shown to be equieffective as a stereotypic agent in both the caudate-putamen and nucleus accumbens although this agent, like dopamine, induced a more intense hyperactivity from the nucleus accumbens. However, these different mechanisms may be preferentially activated by different dopamine agonists. Thus, although the structural relations between the hydroxyl and nitrogen functions in apomorphine are directly comparable to those of dopamine and the primary amine, 5,6-diOHATN, apomorphine was shown to activate the dopamine receptors which mediate stereotyped biting from the caudate-putamen whilst failing to stimulate those dopamine mechanisms in the caudate-putamen *and* nucleus accumbens which mediate hyperactivity. This is in general agreement with the findings of Pijnenburg, Honig & others (1976) who recorded inconsistent effects of apomorphine on activity when injected into the nucleus accumbens. These authors stated that 'little difference was seen between the effects of low and high doses (1 to 10 μg). Both stimulation and depression of activity were sometimes observed in the same animal with different doses of apomorphine'. However, Jackson, Andén & Dahlström (1975) and Grabowska & Andén (1976) reported that a single dose of 10 μg apomorphine stimulated activity on accumbens injection. These data conflict with our observations which suggest that the dopamine mechanisms mediating stereotypy are distinct from those mediating hyperactivity. However, such a differentiation was further confirmed in our studies using *NN*-diPr-5,6-diOHATN which induced stereotypy from both areas, but not hyperactivity.

A further consideration is the possibility of differences in potencies to induce stereotypy from either the caudate-putamen or nucleus accumbens. Thus *NN*-diPr-5,6-diOHATN was active in lower doses in the nucleus accumbens whereas apomorphine and *NN*-diEt-5,6-diOHATN were active in lower doses in the caudate-putamen. However, an absolute comparison of the potencies is made difficult by the differences in the 'amounts' of nucleus accumbens and striatal tissue and the lack of knowledge as to the effective diffusion of agents within the two areas and the precise number of receptor sites activated. Nevertheless, the evidence is suggestive that the mechanisms which mediate stereotypy from the nucleus accumbens (and which are preferentially activated by NPA and *NN*-diPr-5,6-diOHATN) may be different to those in the caudate-putamen (which are preferentially activated by apomorphine and *NN*-diEt-5,6-diOHATN).

In suggesting such differences it is noteworthy that the present study failed to confirm a previous observation that NPA could induce a weak and inconsistent stereotypy from the striatum (Costall & others, 1975a). As well as stereotypy mechanisms, a differentiation of those mechanisms mediating hyperactivity may be indicated since NPA and *NN*-diEt-5,6-diOHATN induced hyperactivity from

the nucleus accumbens but not from the caudate-putamen. However, a more definite differentiation between the mesolimbic and extrapyramidal dopamine mechanisms for hyperactivity induction requires an agent which selectively stimulates those systems which mediate hyperactivity from the caudate-putamen and we have not, as yet, tested such an agent. Nevertheless, the above findings do indicate that a differentiation between hyperactivity mechanisms is a realistic concept.

The idea of different cerebral dopamine mechanisms is not novel, and some attempts have been made to define receptors as DA-1 and DA-2 (Costall & Naylor, 1975) as excitatory and inhibitory (Cools & van Rossum, 1976), but until biochemical and electrophysical evidence is obtained to substantiate the differences reported here, we feel that to attempt such a classification at this stage would be unjustified. Nevertheless, the observation that dopamine agonists are able to selectively stimulate different dopamine systems in different brain regions offers encouragement for the design of agonist drugs to specifically activate discrete dopamine systems and indicates that pharmacological tools are available for the detection of drugs which may specifically block the different extrapyramidal and mesolimbic mechanisms. This has obvious implications for the design of agents to treat disease states associated with a disturbance of dopamine function in the extrapyramidal or mesolimbic system.

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