Differential effects of (3,4-dihydroxyphenylamino)-2-imidazoline (DPI) on hyperactivity responses to dopamine agonists injected into the nucleus accumbens

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The existence of at least two functionally distinct dopamine receptor mechanisms in the nucleus accumbens has been suggested by several workers. One suggestion has been that there is a dopamine mechanism which, when stimulated, can initiate a locomotor hyperactivity (and which is sensitive to neuroleptic blockade), and another whose stimulation (as by DPI) will lead to an inhibition of hyperactivity. These mechanisms have been designated DA_1 and DA_2 respectively (Cools & van Rossum 1976; Cools 1977). Preliminary receptor labelling studies have also indicated the presence of two dopamine binding sites and the 'dopamine-neuroleptic' binding site can be distinguished from the 'DPI-dopamine' binding site (Blackburn et al 1978). At a behavioural level, it could be suggested that any drug which enhances locomotor activity could be acting by DA_1 stimulation or by antagonism of the DA_2 mechanism, and it may be expected that both types of activity would be susceptible to antagonism by a DA_2 agonist such as DPI. In order to investigate this hypothesis we selected four agents which cause marked hyperactivity when injected into the nucleus accumbens, dopamine, (+)-amphetamine, 2-(*NN*-diethyl)amino-5,6-dihydroxy-1,2,-3,4-tetrahydronaphthalene (*NN*-diET-5, 6-di-OHATN) and ergometrine, and determined their interaction with DPI.

Rats were prepared for intra-accumbens injections as previsouly described (Costall & Naylor 1976). Briefly, guide cannulae were stereotaxically implanted for the subsequent insertion of injection units to the centre of the nucleus accumbens (Ant. 9·4, Vert 0·0, Lat. ± 1·6; De Groot 1959). Injections were made into the nucleus accumbens 14 days after surgery: volumes of 1 μl were administered bilaterally over 1 min. Animals were used on one occasion only and at the end of the experiment the brain of every 5th rat was examined histologically to determine the precise point of injection. Injection sites were all confined to the area of the nucleus accumbens (data indistinguishable from that previously reported; Costall & Naylor 1976). Hyperactivity was measured by placing rats in individual cages each fitted with one photocell unit. Interruptions of the light beams were measured electromechanically and hyperactivity expressed in counts per 5 min. All hyperactivity experiments were carried out between 08·00 a.m. and 06·00 p.m. in a sound-proofed, diffusely illuminated room maintained at 21 ± 1 °C.

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Following a 2 h pretreatment with nialamide (Sigma, prepared in a minimum quantity of HCl), 100 mg kg^{-1}, i.p., intra-accumbens dopamine (HCl, Koch Light, prepared in nitrogen bubbled distilled water) induced a dose related hyperactivity (6·25–50 μg): the maximum intensity of effect developed 60–90 min after injection and the duration of effect was in excess of 6 h. A hyperactivity response established to 50 μg dopamine was not modified by 5–40 μg DPI (Boehringer Ingelheim, prepared in distilled water) (Fig. 1). In the absence of nialamide, a dose-dependent hyperactivity was also established to *NN*-diET-5,6-diOHATN (HBr, Cannon, preparation as for dopamine) (1·56–12·5 μg) and, again, an established hyperactivity to this agent (3·13 μg) was resistant to antagonism by 10–40 μg DPI. A transient reduction in the ATN response was recorded during the 2–3 h following the injection of 80 μg DPI (Fig. 1). In contrast to the resistance of the dopamine- and the ATN-induced hyperactivity states to DPI, the hyperactivity responses to intra-accumbens ergometrine (maleate, Sigma) and (+)-amphetamine (SO_4, Sigma) (each prepared in distilled water), induced in the absence of nialamide, were reduced by subsequent intra-accumbens DPI. Amphetamine 0·8–50 μg caused dose-dependent hyperactivity which developed within 15 min and persisted for 2·5–6 h, dependent on the dose. However, the intensity of the hyperactivity induced by ergometrine could not be related to the dose administered, although the duration of response was dose related (6+ h at 1 μg). The hyperactivity responses caused by 6·25 μg amphetamine and 1 μg ergometrine were reduced/abolished by subsequent intra-accumbens injections of 5–10 μg DPI (Fig. 1, all doses expressed as base).

In control experiments it was shown that the hyperactivity responses studied were dopamine-dependent. Thus, the effects of dopamine (50 μg), amphetamine (6·25 μg), the ATN compound (3·13 μg) and ergometrine (1 μg) were specifically reduced/abolished by a dopamine antagonist, fluphenazine, in low doses of 0·2–0·8 mg kg^{-1} i.p., whilst the α- and β-adrenoceptor blocking drugs, piperoxan, 1·25–20 mg kg^{-1} i.p., and propranolol, 1·25–5 mg kg^{-1} i.p., were without effect.

The ability of intra-accumbens dopamine to modulate locomotor activity in the rat has been considered in terms of an ability to increase activity via one dopamine system (DA_1) and to reduce activity via a second system (DA_2) (Cools 1977). The final motor response to intra-accumbens dopamine is hyperactivity, which presumably indicates that the dominant response is DA_1 stimulation: dopamine hyperactivity and therefore

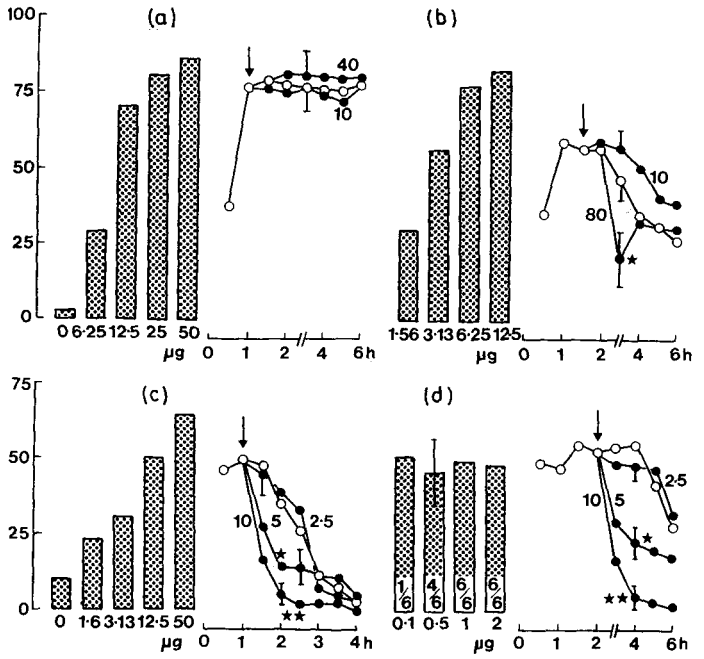

FIG. 1. Hyperactivity induced by intra-accumbens (a) dopamine, (b) *NN*-diEt-5,6-diOHATN, (c) (+)-amphetamine, and (d) ergometrine, and the modification of these hyperactivity responses by intra-accumbens DPI. The histograms indicate the maximum intensity of hyperactivity responses attained throughout the duration of the drug effects. 50 μg Dopamine, 3·13 μg of the ATN compound, 6·25 μg (+)-amphetamine and 1 μg ergometrine were selected to determine the effects of DPI. Control responses to these agonists are indicated as ○—○, and the responses following DPI, administered when hyperactivity was established (↓), are indicated as ●—●. Doses of DPI shown are in μg. n = 6–8. For ergometrine the mean value was derived from the number of animals responding as indicated. Example s.e.m.s are shown. The significance of DPI reversal of locomotor hyperactivity is indicated by $*P < 0{\cdot}01$, $**P < 0{\cdot}001$, (Student's *t*-test).

DA_1 stimulation, is sensitive to neuroleptic blockade (Pijnenburg et al 1975; Jackson et al 1975). This description would also apply to the actions of (+)-amphetamine which releases endogenous dopamine (Jackson et al 1975). However, it is clear that a further action that would result in a hyperactivity could be an inhibition of the DA_2 system and, indeed, ergometrine is purported to be a DA_2 antagonist and has been shown to cause a marked hyperactivity on intra-accumbens injection (Pijnenburg et al 1973; Cools 1977). That this action probably reflects the unchecked activity of the DA_1 system is indicated by the sensitivity of the ergometrine response to neuroleptic blockade (Pijnenburg et al 1973). This concept is further supported by the observations that selective stimulation of the DA_2 system using DPI will reduce the hyperactivity responses to both ergometrine and amphetamine (Cools 1977, present studies), the antagonism of the ergometrine effect essentially reflecting an agonist-antagonist interaction at the DA_2 receptor, and the antagonism of amphetamine reflecting an indirect modulation of the DA_1 system function via the DA_2 mechanism. Clearly, these interpretations should be extrapolated to dopamine itself, and DPI may be expected to antagonize the hyperactivity caused by intra-accumbens dopamine. However, the present results show that DPI does *not* reduce dopamine hyperactivity. It may be argued that this observation reflects an ability of nialamide, used in combination with dopamine, to exaggerate the effects of dopamine on the DA_1 system and reduce the dopamine agonist action of DPI on the DA_2 system (see Cools 1977), but the hyperactivity induced by the ATN compound, in the absence of nialamide, was also virtually unaffected by DPI. This ATN compound has been shown to bind to neuroleptic receptors, and its action in the nucleus accumbens to cause hyperactivity is sensitive to neuroleptic blockade (Cannon et al 1978; Costall et al 1977). Therefore, the failure of DPI to antagonize the ATN hyperactivity should be considered in the light of this evidence that the ATN can affect a DA_1 site. Thus, it is concluded that the inhibitory system activated by DPI (DA_2 system) does not necessarily exert absolute control over the function of the DA_1 system.

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On the disintegration of hard gelatin capsules in fasting volunteers using a profile scanning technique

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Hard gelatin capsules have become a common and useful oral dosage form for drugs. Several studies on the influence of formulation factors on the in vitro dissolution behaviour of such capsules have been published (Newton 1972, Newton et al 1971, Newton & Razzo 1974). Attention has also been drawn to the influence of the gelatin capsule on the bioavailability of a drug (Kranz et al 1977). However, there seems to be limited information available on disintegration in vivo. This may partly be due to the difficulty in performing such in vivo tests and the lack of useful methods. In an often cited study, Eckert (1976) used capsules filled with $NaHCO_3$ and determined the disintegration time by the change in pH of the gastric juice. He found that the content was released in 2·5 to 6 min. Recently, a new method using ^{99}Tcm and external scintigraphy was presented (Casey et al 1976). The study was based on few observations, but the authors reported a disintegration of 30–40 min for a capsule with an insoluble content and 6 min for a capsule containing a more soluble formulation.

In this communication we present data on the in vivo disintegration of gelatin capsules in fasting humans using a profile scanning technique (Alpsten et al 1976). Two different acetylsalicylic acid (ASA) formulations were studied. Formulation 1 consisted of 500 mg of ASA granules, 0·5–1 mm, dispensed in a hard gelatin capsule (Capsugel size 0+). Formulation 2 was similar to the first one but the granules were coated with an acid-resistant film (5% ASA was released in 2 h using the U.S.P. rotating basket procedure, 150 rev min^{-1} 0·1 M HCl). The ASA granules were marked by granulation with a radionuclide ^{51}Cr (1·85 MBq/50 μCi). The radiation was measured externally by using a moveable detector in a low activity laboratory (Sköldborn et al 1972). The method permitted determination of the position and distribution of the source within the body. The capsules were considered to have disintegrated when the point source (^{51}Cr) changed from the approximate punctate shape to a broadly distributed source (Alpsten et al, be published).

The subjects, who had fasted for 10 h, swallowed one capsule together with 100 ml of water following a cross-over design. During the measurements the volunteers were sitting in an armchair in a well-defined position while the detector was scanned back and forth repeatedly. After four such scans, corresponding to about 15 min, a break of about 5 min was made and the volunteer was allowed to move about freely. The complete 20 min cycle was repeated as long as was needed to register the disintegration of the capsule.

The disintegration time of the capsules in vitro was 1–2 min according to the official method of the British Pharmacopoeia 1973 and 2–4 min according to the method of Pharm. Nord. There was no difference in the in vitro disintegration time between the two formulations. The in vivo results are summarized in Table 1 and show that the disintegration, i.e. dispersion in the stomach, is slower than that observed in the two in vitro tests. There was an interindividual variation in the in vivo disintegration time of the capsules of 8–25 min for formulation 1 and 12–20 min for formula-

Table 1. Disintegration time of gelatin capsules in vivo

Subject	Formulation 1 ASA-granules	Formulation 2 enteric-coated ASA-granules
1	25	20
2	8	26
3	20	12
4	8	12
5	—	20
6	12	20
Mean	15	18

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