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Involvement of mesolimbic and extrapyramidal nuclei in the motor depressant action of narcotic drugs

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The ability of narcotic drugs to induce motor depression from the mesolimbic nucleus accumbens (ACB) and extrapyramidal caudate-putamen (CP) was investigated using the bilateral intracerebral injection technique. Fluphenazine and procaine were used as control agents. Firstly, drugs were injected alone into the ACB or CP and catalepsy was assessed. Fentanyl (2·5–10 μg), sufentanil (0·25–1 μg) and carfentanil (0·05–0·5 μg) were shown to be potent cataleptogens when injected into both the ACB and CP, the responses being dose-dependent and achieving maximum intensity. Morphine (1–50 μg) also induced a marked dose-dependent catalepsy, but only after injection into the ACB. In contrast, pethidine and methadone, in doses up to 160 μg, caused only weak and inconsistent responses from the ACB and CP. Similar injections of procaine (50–200 μg) were ineffective, but fluphenazine (25–200 μg) induced a moderate dose-dependent response from both the ACB and CP, although onset of action was more rapid and the duration markedly longer for the latter injections. Secondly, drugs were injected peripherally and intracerebrally to determine their ability to antagonize the marked hyperactivity induced by intra-ACB dopamine in the presence of nialamide. Two agents shown to induce catalepsy from the ACB, fluphenazine and morphine, antagonized dopamine hyperactivity when they were administered peripherally or directly into the ACB (0·1–0·2 mg kg^{-1}, i.p. or 3·1–25 μg fluphenazine and 1–5 mg^{-1} kg, s.c. or 1–5 μg morphine), but only a weak antagonism occurred at much larger doses after intra-CP injections (100 μg fluphenazine and 50 μg morphine). Larger doses of intra-ACB pethidine (160 μg) and procaine (100–200 μg) also antagonized the dopamine response, but methadone was inactive. Again, the most potent and effective drugs in this test were fentanyl (1–5 μg), sufentanil (0·25–0·5 μg) and carfentanil (0·05–0·1 μg). It is suggested that the ACB, and not the CP, is the site at which morphine acts to cause motor depression, whilst other narcotic drugs are able to act in both areas (although there is some indication of a further unspecified site of action for methadone). In contrast, the neuroleptic fluphenazine appears to differentially affect motor function via the ACB and CP, antagonizing a dopamine hyperactivity in the former and primarily inducing catalepsy from the latter nucleus.

Morphine has been shown to reduce or enhance motor function depending on the dose administered, the duration of treatment and the species used. In the rat, its immediate effect, in moderate doses, is to induce motor depression and an immobility described as catalepsy or catatonia (Kuschinsky & Hornykiewicz, 1972; Costall & Naylor, 1973a). Numerous studies have implicated an inhibition of striatal (CP) dopamine function with drug-induced cataleptic behaviour (see review by Fog, 1972) and a CP site of action for morphine catalepsy in the rat has been presumed on the basis that morphine will modify dopamine turnover (Kuschinsky & Hornykiewicz, 1972, 1974). However, CP lesions which are known to reduce neuroleptic catalepsy do not reduce morphine catalepsy (Fog, 1972; Costall & Naylor, 1973b). Furthermore, recent studies have indicated that the nucleus accumbens (ACB) may provide a potential site for morphine to induce behavioural depression (Costall, Fortune & Naylor, 1976, 1977; Dill & Costa, 1977).

Therefore, in the present study we have used the intracerebral injection technique to compare the cataleptic actions of morphine and the potent dopamine receptor blocking agent, the neuroleptic fluphenazine, when injected into mesolimbic (ACB) and striatal (CP) areas, and have determined their ability to antagonize the hyperactivity induced by dopamine injected into the ACB. The specificity of the findings with morphine to the narcotic drugs was further assessed by using a range of such agents in the same test situations.

MATERIALS AND METHODS

Animals

Male, Sprague-Dawley (CFE) rats, between 300–350 g at the time of operation, were used. The weight range was chosen since the head growth of animals weighing >300 g is minimal and not sufficient to displace cannulae or to detach the holder from the

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skull. Animals were housed in groups of 8 and had free access to food and water.

Intracerebral injection technique

Guide cannulae (0·65 mm external diameter) fixed in Perspex blocks were implanted in the brains of animals anaesthetized with chloral hydrate (300 mg kg^{-1}, i.p.). These cannulae were kept patent by the use of stylets (0·3 mm diameter), and all animals were allowed at least 7 days to recover from the operation before use.

Following withdrawal of the stylet, insertion of an injection unit (0·3 mm diameter) attached to an Agla micrometer syringe allowed deposition of solvent (distilled water) or drug solution into the area under investigation. The injection units projected either 2·5 mm or 1·5 mm beyond the tips of the appropriate guide cannula for injection into the ACB (Ant. 9·4, Vert. 0·0, Lat. ±1·6) or CP (Ant. 8·2, Vert. +1·5, Lat. ±3·0) respectively (the atlas of De Groot, 1959, was used as a guide).

All intracerebrally injected agents were freshly prepared and administered immediately in a dose volume of 1 μl. All injections were bilateral and doses, expressed as the base, refer to the unilateral quantity. Immediately after injection animals were replaced into individual boxes for behavioural observations.

Histological techniques

Upon completion of the studies the location of the injection sites were confirmed using the techniques of Costall, Fortune & Naylor (1978).

Assessment of catalepsy

For observation and assessment of catalepsy, animals were placed in individual Perspex cages (30 × 15 cm and 15 cm high) equipped with a 10 cm high horizontal bar. Animals were tested by placing both front limbs over the bar, a cataleptic animal maintaining this abnormal position for a period of time dependent on the degree of catalepsy; this time was converted to a numerical score as shown in Table 1. The intensity of cataleptic behaviour was assessed frequently after drug administration to determine the onset and subsequently at 5–15 min intervals throughout the duration of drug effect.

In the present extensive experiments there was no indication of a learning phenomenon in the catalepsy testing, the maximum intensity generally being maintained at a constant value throughout a multi-test period. This is demonstrated particularly by animals allocated a low intensity score for catalepsy: this score was not increased by a series of subsequent tests. For this reason the onset of catalepsy, the maximum score produced, and the duration of this behaviour are shown in the results section (Table 2).

Table 1. *Scoring system used for the assessment of catalepsy.*

Intensity (min)	Score
0	0
0·1–2·5	1
2·6–5·0	2
5·1–10·0	3
10·1–20·0	4
20·1–∞	5

Measurement of activity

For measurement of activity animals were transferred to similar individual Perspex cages equipped with photoelectric cells. The number of interruptions, by the rat, of a light beam passing through the cage was recorded and the activity expressed according to the number of counts per 5 min period. Visual interaction between the rats was prevented by use of screens between the cages.

Experimental design

All observations were made between 09·00 and 20·00 h. Animals were used on no more than 3 occasions with at least 7 days between treatments No change in locomotor activity occurred as a result of animals being exposed to the recording apparatus on previous occasions.

Statistical analyses

The distribution of values obtained in the hyperactivity and catalepsy experiments were sufficiently close to normal to allow the application of parametric statistics. The significance of the results was assessed by using the Student's *t*-test.

Drugs

Nialamide (Sigma) was dissolved in the minimum quantity of hydrochloric acid and made up to volume with distilled water. Fentanyl citrate, sufentanil citrate and carfentanil citrate [methyl-4-[*N*-(1-oxopropyl)-*N*-phenylamino]-1-(2-phenylethyl)-4-piperidinecarboxylate-2-hydroxy-1,2,3-propanetricarboxylate(1:1)] (Janssen), fluphenazine hydrochloride (Squibb), methadone hydrochloride (Burroughs Wellcome), morphine hydrochloride (BDH), pethidine hydrochloride and procaine hydrochloride (May & Baker) were dissolved in distilled water.

Table 2. *Catalepsy induced by intracerebral drug administration into the ACB and CP. Each value is the mean response of 6–8 rats together standard error of the mean.*

	Intra-ACB				Intra-CP			
Agent	Dose μg	Onset (min)	Max catalepsy score	Duration (min)	Dose μg	Onset (min)	Max catalepsy score	Duration (min)
Vehicle (distilled water)	1 μl	—	0·0 ±0·0	0·0 ±0·0	1 μl	—	0·0 ±0·0	0·0 ±0·0
Fluphenazine	25		0·0 ±0·0	0·0 ±0·0	25	14·6 ±2·6	1·0 ±0·0	233·3 ±48·9
	50		0·0 ±0·0*	0·0 ±0·0	50	9·7 ±1·6	1·3 ±0·2	355·0 ±32·3
	100	43·1 ±10·0	1·5 ±0·2	375·0 ±22·7	100	6·9 ±0·9	2·0 ±0·3	435·0 ±12·9
	200	58·1 ±7·7	2·1 ±0·2	405·0 ±24·1				
Morphine	1	10·6 ±1·6	1·0 ±0·0	72·5 ±8·4	1	—	0·0 ±0·0	0·0 ±0·0
	3·1	9·4 ±1·4	1·6 ±0·2	103·1 ±2·9	3·1	—	0·0 ±0·0	0·0 ±0·0
	12·5	7·3 ±2·2	2·8 ±0·2	151·7 ±7·2	12·5	—	0·0 ±0·0	0·0 ±0·0
	50	4·6 ±0·9	4·7 ±0·2	162·9 ±10·2	50	—	0·0 ±0·0	0·0 ±0·0
Procaine	50	—	0·0 ±0·0	0·0 ±0·0	50	—	0·0 ±0·0	0·0 ±0·0
	100	—	0·0 ±0·0	0·0 ±0·0	100	—	0·0 ±0·0	0·0 ±0·0
	200	—	0·0 ±0·0	0·0 ±0·0	200	—	0·0 ±0·0	0·0 ±0·0
Pethidine	20	—	0·0 ±0·0*	0·0 ±0·0				
	40	—	0·0 ±0·0*	0·0 ±0·0	40	—	0·0 ±0·0	0·0 ±0·0
	80	—	0·0 ±0·0*	0·0 ±0·0	80	—	0·0 ±0·0*	0·0 ±0·0
	160	9·4 ±1·8	1·4 ±0·2	46·3 ±8·3	160	—	0·0 ±0·0*	0·0 ±0·0
Methadone	20	—	0·0 ±0·0*	0·0 ±0·0				
	40	—	0·0 ±0·0*	0·0 ±0·0	40	—	0·0 ±0·0	0·0 ±0·0
	80	—	0·0 ±0·0*	0·0 ±0·0	80	—	0·0 ±0·0	0·0 ±0·0
	160	—	0·0 ±0·0*	0·0 ±0·0	160	—	0·0 ±0·0*	0·0 ±0·0
Fentanyl	1	—	0·0 ±0·0*	0·0 ±0·0				
	2·5	2·3 ±0·3	2·8 ±0·2	36·5 ±7·0	2·5	4·4 ±0·5	1·7 ±0·2	18·3 ±3·8
	5	2·6 ±0·4	3·7 ±0·2	40·8 ±5·1	5	3·7 ±0·7	3·2 ±0·3	35·0 ±5·5
	10	1·6 ±0·2	5·0 ±0·0	90·0 ±4·9	10	2·5 ±0·3	5·0 ±0·0	72·5 ±5·1
Sufentanil	0·1	—	0·0 ±0·0*	0·0 ±0·0	0·05	—	0·0 ±0·0*	0·0 ±0·0
	0·25	4·7±0·7	1·0 ±0·0	26·7 ±5·6	0·25	5·6 ±1·2	2·8 ±0·3	37·5 ±6·2
	0·5	3·2 ±0·4	3·5 ±0·2	87·5 ±3·8				
	1	3·1 ±0·4	5·0 ±0·0	105·0 ±5·7	1	2·6 ±0·2	5·0 ±0·0	87·5 ±3·4
Carfentanil	0·01	—	0·0 ±0·0*	0·0 ±0·0				
	0·05	3·5 ±1·0	1·3 ±0·2	65·6 ±3·2	0·05	—	0·0 ±0·0*	0·0 ±0·0
	0·1	3·6 ±0·3	3·8 ±0·2	76·4 ±2·4	0·1	9·5 ±0·8	2·3 ±0·3	42·5 ±4·0
	0·5	2·8 ±0·3	5·0 ±0·0	83·8 ±3·8	0·5	7·2 ±1·0	5·0 ±0·0	75·7 ±2·5

* 50% or less of animals tested responded with a score 1 catalepsy.

Dopamine hydrochloride (Koch–Light) was dissolved in nitrogen bubbled distilled water.

RESULTS

Histological assessment of injection sites

All injection sites were found to be within the areas under investigation (Fig. 1).

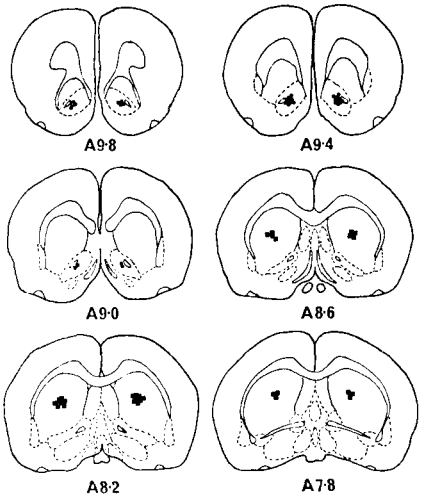

FIG. 1. Diagrammatic representation of the location of the sites of injection into the nucleus accumbens (●) and caudate-putamen (■), coordinates according to De Groot (1959). The diagrams were constructed from the histological data from 8 typical brains of each group of rats receiving either intra-ACB, intra-CP, or intra-ACB + intra-CP injections.

Catalepsy observed after intracerebral drug administration

Fluphenazine (25–100 μg) rapidly induced (7–15 min) a long-lasting, moderately intense catalepsy following injection into the CP, whereas its application to the ACB (100–200 μg) produced a similar intensity response after a much longer latency of onset (40–60 min). Also, the duration of action was significantly longer for intra-CP injections (Table 2). In contrast, morphine (1–50 μg) caused a marked dose-dependent catalepsy, associated with normal or increased body tone, only after intra-ACB injection; this behaviour developed within 5–10 min and lasted for 70–160 min. Pethidine (20–160 μg) and methadone (20–160 μg) produced only a weak or irregular response whereas procaine (50–200 μg) was inactive after injection into both the CP and ACB. However, fentanyl (2·5–10 μg) and its derivatives sufentanil (0·25–1 μg) and carfentanil (0·05–0·5 μg) were potently cataleptogenic following administration to either brain site (Table 2). The catalepsy evoked by these narcotic drugs had a rapid onset (1·5–10 min) and dose-related duration (5–90 min), and was associated with pronounced body rigidity.

Dopamine induced hyperactivity

The bilateral injection of dopamine (5–100 μg) into the ACB of rats pretreated (2 h) with nialamide

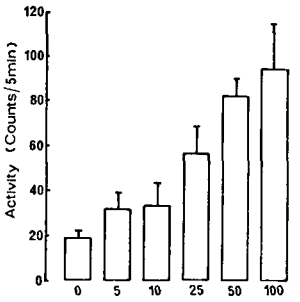

FIG. 2. Maximum activity, expressed in counts/5 min (ordinate), produced by 5–100 μg dopamine or vehicle control administered bilaterally into the ACB of rats pretreated for 2 h with nialamide (100 mg kg^{-1}, i.p.). For all doses of dopamine causing a significant hyperactivity response, onset was within 30 min and the maximum response was maintained for 3·5–8 h. Each value is the mean response of 8–10 rats together with standard error of the mean.

(100 mg kg^{-1}, i.p.) induced a dose-related increase in locomotor activity (Fig. 2). This response attained a maximal effect after 90–120 min which persisted for a further 4–5 h.

Modification of the effects of intra-ACB dopamine

All agents were administered to animals with an established hyperactivity 150 min after dopamine (50 μg) was injected into the ACB. Peripheral (0·1–0·2 mg kg^{-1}, i.p.) or intra-ACB (3·1–25 μg) injections of fluphenazine significantly ($P < 0·05$–$P < 0·001$) antagonized the hyperactivity response. This antagonism persisted for the duration of the dopamine effect. However, the deposition of much larger doses of fluphenazine (100 μg) into the CP was required to decrease ($P < 0·05$) the hyperactivity induced by intra-ACB dopamine. Similarly, the administration of morphine, either peripherally (1–5 mg kg^{-1}, s.c.), into the ACB (1–5 μg) or, in a comparatively large dose, into the CP (50 μg) was effective in inhibiting ($P < 0·05$–$P < 0·001$) the hyperactivity. However, whilst the antagonism by

both fluphenazine and morphine applied peripherally or directly to the ACB was rapid in onset, following intra-CP injection a latency of 2–3 h was recorded before a significant decrease in activity was produced (Fig. 3).

Large doses of intra-ACB procaine (100–200 μg) and pethidine (160 μg) were required to significantly reduce the dopamine-induced hyperactivity, and methadone, in doses up to 80 μg, was inactive in this respect. However, fentanyl (1–5 μg), sufentanil (0·25–0·5 μg) and carfentanil (0·05–0·1 μg) showed potent ability to decrease ($P < 0·05$–$P < 0·001$) the dopamine hyperactivity. The antagonism caused by these agents was rapid in onset; but the dopamine response recovered within the 3·5 h test period (Fig. 4).

DISCUSSION

In studies on the effects of intra-striatal morphine, evidence for a cataleptic action is conspicuously absent (Jacquet & Lajtha, 1973; Baker, Lalley & Young, 1974; Broekkamp & van Rossum, 1975; Lalley, Rossi & Baker, 1975; Yaksh, Yeung & Rudy, 1976). In contrast, Bergmann, Chaimovitz & others (1974) reported the development of a weak gnawing response in rats and, similarly, the induction of a weak biting and hyperactivity was observed in the present experiments and reported earlier (Costall & others, 1978). Intrastriatal dopamine can induce similar effects and we therefore consider that the changes in striatal dopamine turnover by peripheral morphine treatment may be related to the motor stimulant potential of morphine rather than a

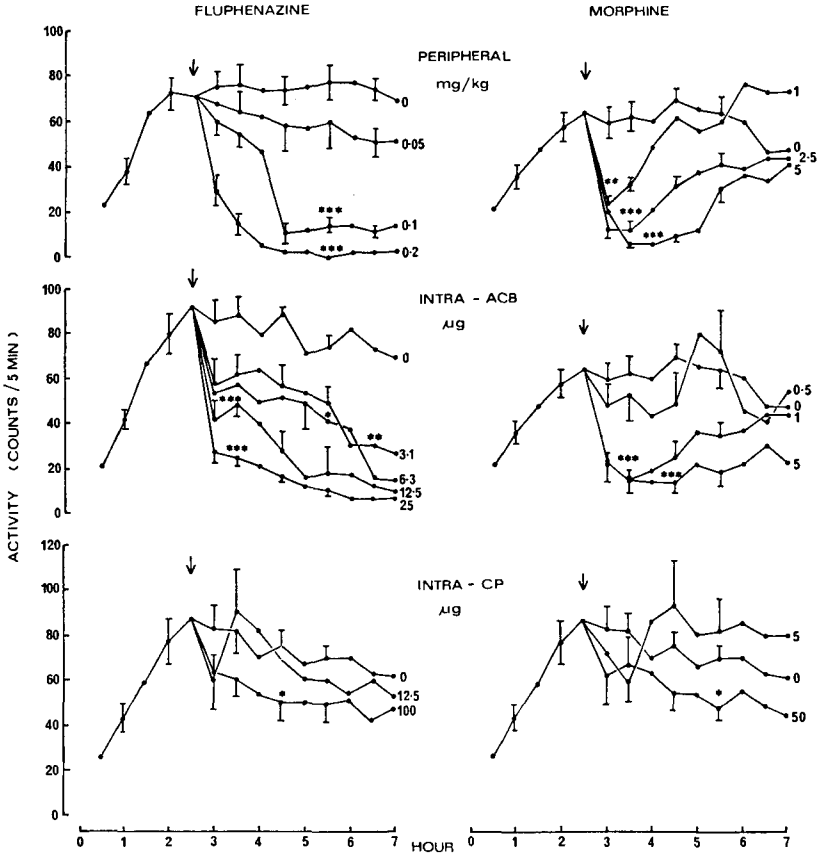

FIG. 3. Inhibitory effects of fluphenazine and morphine on the hyperactivity caused by 50 μg dopamine applied to the ACB. Arrows indicate the time of administration of fluphenazine or morphine peripherally (expressed in mg kg^{-1} i.p. or s.c. respectively), into the ACB (μg), or into the CP (μg). Each value is the mean response of 6–10 rats and diagrams show representative standard errors of the mean. Significant differences compared with control values are indicated by * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$. Ordinate: Activity (counts/ 5 min). Abscissa: Time (h).

cataleptic action. This is in accordance with the interpretation from striatal lesion studies where electrolytic lesions of the neo- and paleo-striatum were found to enhance the cataleptic action of morphine (Costall & Naylor, 1973b), and where it was concluded that narcotic drug action within the striatal areas may oppose the development of catalepsy. More discrete lesioning of the striatal dopamine system (intra-striatal 6-hydroxydopamine) similarly enhances morphine catalepsy (Nakamura, Kuntzman & others, 1973). We would, however, emphasize that morphine can induce motor stimulant action on injection into other brain areas, the tuberculum olfactorium, area preoptica and peri-aqueductal grey (Jacquet & Lajtha, 1974; Costall & others, 1978). The CP may, therefore, not be an exclusive site for the stimulant actions of morphine.

Although the injection of morphine into the CP fails to induce catalepsy, this behaviour can be induced by intra-ACB administration, and control injections into surrounding areas indicate that the response observed is due to an action within this nucleus (Costall & others, 1978). It is possible that the very potent and specific narcotic drugs fentanyl, sufentanil and carfentanil (Janssen, unpublished data; Niemegeers, Schellekens & others, 1976; Leysen, Tollenaere & others, 1977) may have a similar site of action since their cataleptic effect is very rapid in onset following injection into this mesolimbic area, and the intensity of response and

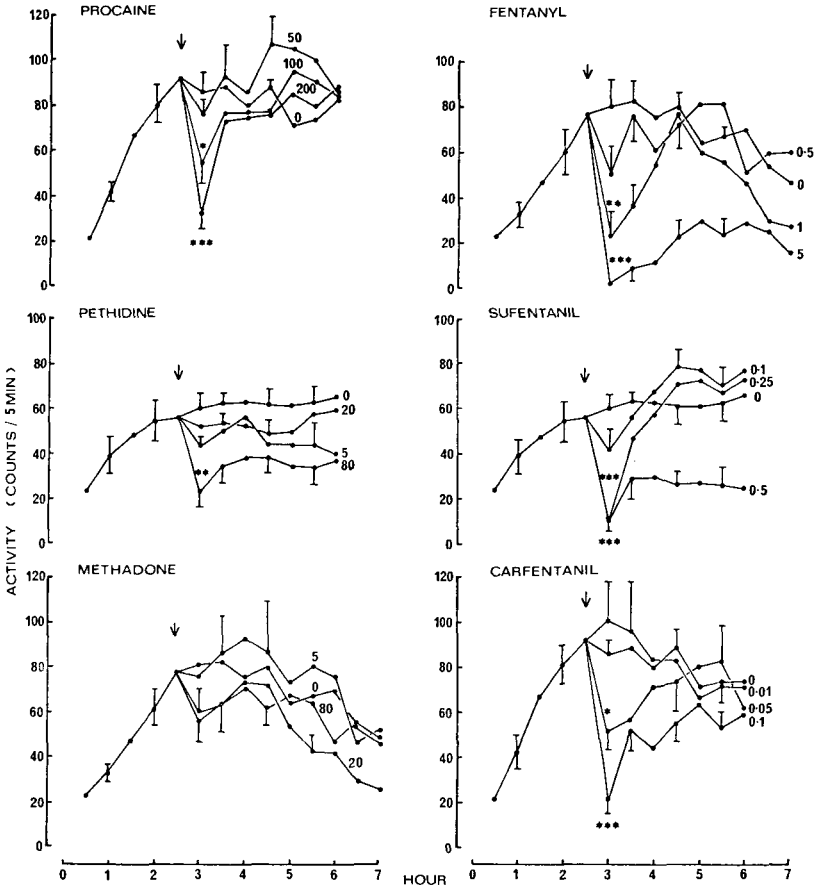

FIG. 4. Modification by intra-accumbens procaine, pethidine, methadone, fentanyl, sufentanil and carfentanil of the hyperactivity caused by 50 μg dopamine applied to this nucleus. The doses are expressed in μg and control animals received the vehicle (1 μl distilled water). Arrows are used to indicate the time of injection of the agents. Each value is the mean response of 6–8 rats, and the diagrams show representative standard errors of the means. Significant differences from control values are indicated by * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$. Ordinate: Activity (counts/5 min). Abscissa: Time (h).

its duration are dose-related. That these agents may be affecting specific receptor mechanisms within the ACB is further emphasized by the ineffectiveness of procaine. Similar injections of fluphenazine into the ACB evoked only a weak response after a long latency of onset, and in doses approximately one thousand fold greater than carfentanil. Since fluphenazine is considered a highly potent and specific dopamine receptor blocking agent, the lack of an effective response would suggest that blockade of dopamine mechanisms within the ACB is not in itself sufficient to induce marked catalepsy.

Nevertheless, both morphine and fluphenazine administered either peripherally or directly into the ACB were shown to decrease the motor stimulant effects of intra-ACB dopamine, emphasizing that the dopamine function in this mesolimbic nucleus is critically involved in motor control. Potent dopamine inhibitory effects were also produced by intra-ACB fentanyl and its derivatives. The locus specificity of antagonism is indicated by the failure of intra-CP morphine and fluphenazine to decrease the mesolimbic hyperactivity response except in much higher doses when diffusional effects may be prominent, particularly since 2–3 h were required before these agents were found to exert any significant effect. Therefore, whilst fluphenazine is a highly effective antagonist of the hyperactivity induced by intra-ACB dopamine, and agents such as sufentanil and carfentanil are 10–100 times more potent, it is further apparent that the potent narcotic drugs must exert some effects additional to their ability to decrease dopamine function in order to precipitate cataleptic behaviour following injection into the ACB.

The present findings do not allow an extension of the hypothesis of a locus specificity of mesolimbic action shown by morphine to other narcotic drugs, for fentanyl, sufentanil and carfentanil produced an effective cataleptic response both on intra-CP as well as intra-ACB injection. For the cataleptic action of these and other narcotic drugs, both mesolimbic and extrapyramidal sites of action should be considered, and the virtual failure of methadone, an agent with known cataleptic action on peripheral injection, to induce this behaviour after deposition into either the ACB or CP may indicate further, non-specified site(s) of cataleptic action.

In summary, the inability of morphine to induce catalepsy on intra-CP injection does not support an extrapyramidal site for morphine catalepsy, whilst other narcotic drugs such as fentanyl, sufentanil and carfentanil may partially induce their cataleptic effects by an action within the extrapyramidal system. In contrast to this differential role in the extrapyramidal system, both morphine and fentanyl and its derivatives induced catalepsy on intra-ACB injection and antagonized the hyperactivity induced by intra-ACB dopamine. The ability, and potency, of these agents to modify dopamine function indicate their potential as tools in elucidating striatal and mesolimbic mechanisms involved with motor control.

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