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Dopamine receptor binding sites in the rat superior colliculus

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Following intravenous administration of [^{3}H]spiperone or [^{3}H]*N*,n-propylnorapomorphine (NPA) to rats, radioactivity derived from the ligands accumulated in the striatum and superior colliculus when compared with cerebellar levels. The accumulation of [^{3}H]spiperone in both areas was prevented by intraperitoneal administration of (+)-butaclamol, haloperidol and sulpiride but not by (−)-butaclamol, cinanserin, propranolol or prazosin. The accumulation of [^{3}H]NPA was prevented by administration of (+)-butaclamol, haloperidol and apomorphine but not by (−)-butaclamol. In in-vitro experiments, membrane preparations from the superior colliculus showed a small number of specific binding sites for both [^{3}H]spiperone and [^{3}H]NPA. The dissociation constant (K_D) for [^{3}H]NPA was not different from that for striatal preparations but that for [^{3}H]spiperone was 10-fold higher. We conclude that dopamine receptors may be present within the superior colliculus.

The superior colliculus aids visuomotor coordination by controlling the orientation of the head and eyes towards external stimuli (Sprague & Meikle 1965; Kirvel et al 1974; Sprague & Ingle 1975; Wurtz & Albano 1980). It receives efferent fibres from the zona reticulata of the substantia nigra (Graybiel & Sciascia 1975; York & Faber 1977) and has been suggested to be involved in the mediation of dopamine-dependent behaviours such as circling or stereotypy initiated from the striatum (Pope et al 1980; Redgrave et al 1980; Morelli et al 1981; Kilpatrick et al 1982). The nigrotectal pathway is thought to utilize GABA as a neurotransmitter (Di Chiara et al 1979; Kilpatrick et al 1982). We were therefore surprised to find during the course of an investigation of the in-vivo binding of [^{3}H]spiperone and [^{3}H]*N*,n-propylnorapomorphine that radioactivity derived from them accumulated in the superior colliculus. We have attempted to characterize the nature of these binding sites and present evidence to suggest the presence of dopamine receptors in the superior colliculus.

Materials and methods

In-vivo bindings assay. [^{3}H]Spiperone (25 Ci mmol^{-1}; 25 μCi equivalent to 5·0 μg kg^{-1}; Amersham International) or (−)-[^{3}H]*N*,n-propylnorapomorphine ([^{3}H]NPA) (58·5 Ci mmol^{-1}; 25 μCi equivalent to 0·8 μg kg^{-1}; New England Nuclear) were administered in 0·25 ml 0·9% w/v NaCl (saline) via the tail vein to female Wistar rats (150 ± 10 g; Bantin & Kingman). Rats were killed 60 min after administration of [^{3}H]spiperone or 10 min after [^{3}H]NPA. In some experiments animals also received before death apomorphine hydrochloride (MacFarlan Smith) (0·5 mg kg^{-1} s.c., 15 min before), (+)- or (−)-butaclamol (Ayerst Laboratories) (5 mg kg^{-1} i.p., 30 min before), haloperidol (Janssen Pharmaceutica) (1·0 mg kg^{-1} i.p., 60 min before), (±)-sulpiride (Delagrange) (50 mg kg^{-1} i.p., 180 min before), propranolol hydrochloride (ICI) (20 mg kg^{-1} i.p., 120 min before), prazosin hydrochloride (Pfizer) (1 mg kg^{-1} i.p., 120 min before) or cinanserin hydrochloride (Squibb) (5 mg kg^{-1} i.p., 90 min before). The timing of drug administration and doses of unlabelled drug used in competition experiments were chosen as being pharmacologically active in behavioural tests. Animals were killed by cervical dislocation and decapitation, then the brain was rapidly placed on ice, the areas carefully dissected, frozen at −20 °C and weighed before being stored at −70 °C until analysis. The dissection was based on Glowinski & Iversen (1966) as follows: the brain was positioned with its dorsal surface uppermost and the cerebellum was removed using iris forceps. The two hemispheres of the forebrain then were separated to expose the corpus striatum and superior colliculus. The corpus striatum (consisting of putamen, caudate nucleus and globus pallidus) and the superior colliculus were then removed using iris forceps.

Tissue samples were oxidized using a Packard oxidizer b306, the resulting ^3H_2O trapped and counted at an efficiency of 40–45% using a Packard 460C scintillation counter. Counts were transformed to d min^{-1} to allow for differences in counting efficiency and expressed in terms of mg of tissue. Radioactivity accumulated in the striatum or superior colliculus was compared with radioactivity in the cerebellum, an area thought to contain no dopaminergic innervation. Prevention or displacement of accumulated radioactivity was compared with tissue from animals that received ligand alone.

Following removal of the superior colliculus the brains of some animals were fixed in 40% formaldehyde–glacial acetic acid–methanol (1:1:8) for one week. Serial paraffin sections were cut at 20 μm and stained with cresyl fast violet. Sections were examined using a Projectina projecting microscope.

In-vitro binding experiments. Striatal and superior colliculus tissue for in-vitro binding experiments was

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removed from untreated Wistar rats as described for in-vivo procedures and was stored at −20 °C until required. Membrane fractions for [^{3}H]spiperone binding were prepared as previously decribed (Leysen et al 1978) except that final homogenization and incubation was in Tris-HCl 50 mM containing 120 mM sodium chloride. Tissue for [^{3}H]NPA binding was prepared as previously described (Leysen & Gommeren 1981) except that all procedures were performed in a Tris-EDTA buffer (Hall et al 1981). Specific [^{3}H]spiperone (0·1–4·0 nM) binding was defined using (−)-sulpiride (10^{-5} M) or (+)-butaclamol (10^{-6} M); specific [^{3}H]NPA (0·05–2·0 nM) binding was defined using (+)-butaclamol (10^{-5} M) or (±)-ADTN (10^{-6} M; Wellcome Research Laboratories). Data from at least six ligand concentrations examined in triplicate was subjected to Scatchard analysis, followed by linear regression analysis to give estimates of the number of binding sites (B_{max}; pmoles g^{-1} tissue) and equilibrium dissociation constant (K_D; nM). Estimates from six independent determinations each carried out using pooled tissue from 12 animals were averaged to provide a mean ± s.e.m.

Results

In-vivo binding experiments. Following intravenous administration of [^{3}H]spiperone (25 μCi), radioactivity derived from the ligand accumulated in the striatum and superior colliculus, when compared with cerebellar levels (Fig. 1). Administration of (+)-butaclamol (5 mg kg^{-1} i.p. 30 min before death), but not of the inactive (−)-isomer, prevented the accumulation of [^{3}H]spiperone in the striatum and superior colliculus but did not alter cerebellar levels of radioactivity. Administration of haloperidol (1 mg kg^{-1} i.p., 60 min before death) or (±)-sulpiride (40 mg kg^{-1} i.p., 180 min before death) also prevented the accumulation of radioactivity derived from [^{3}H]spiperone in the striatum and superior colliculus, but again did not affect cerebellar levels. Administration of the 5-HT antagonist cinanserin, or the adrenergic antagonists propranolol and prazosin, did not alter the accumulation of radioactivity derived from [^{3}H]spiperone in any area.

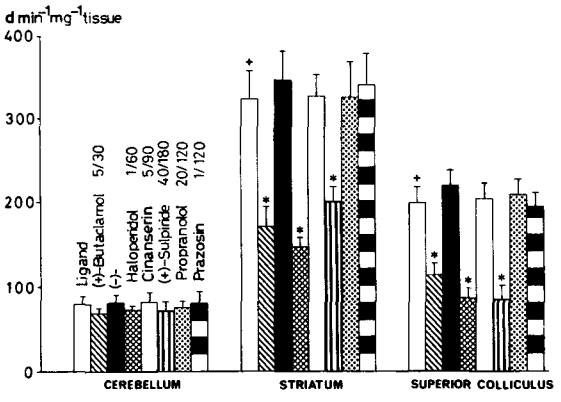

FIG. 1. The accumulation of radioactivity derived from [^{3}H]spiperone (25 μCi i.v.) in striatum, superior colliculus and cerebellum of rat brain and its prevention by administration of (+)- or (−)-butaclamol, haloperidol, cinanserin, (±)-sulpiride, propranolol or prazosin. †$P < 0.05$ compared with cerebellar levels; Student's t-test. *$P < 0.05$ compared with accumulation occurring in the same area in the absence of unlabelled drugs; Student's t-test. The code is as shown for cerebellum, with dose in mg kg^{-1} and time in min before death. Each value is the mean (±1 s.e.m.) of the radioactivity measured in tissue from at least 8 animals.

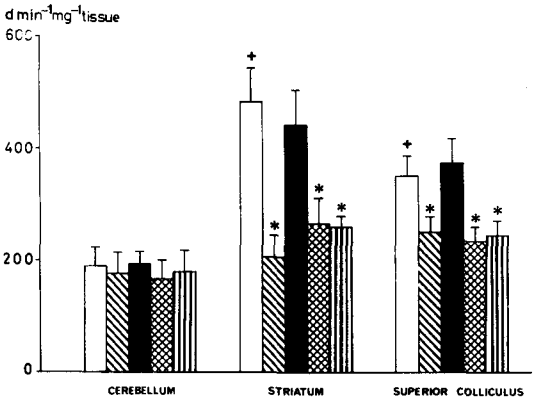

FIG. 2. The accumulation of radioactivity derived from [^{3}H]*N*,n-propylnorapomorphine (25 μCi i.v.) in striatum, superior colliculus and cerebellum of rat brain and its prevention by administration of (+)- or (−)-butaclamol, haloperidol (coded as shown in Fig. 1), or apomorphine, 0·5 mg kg^{-1} s.c. 15 min before death as column with vertical hatching. †$P < 0.05$ compared to cerebellar levels. *$P < 0.05$ compared to accumulation occuring in the same area in the absence of unlabelled drug. Each value is the mean (±1 s.e.m.) of the radioactivity measured in tissue from at least 8 animals.

After i.v. administration of [^{3}H]NPA, radioactivity derived from the ligand accumulated over cerebellar levels in the striatum and superior colliculus (Fig. 2). The accumulation was prevented by apomorphine (0·5 mg kg^{-1} s.c., 15 min before death), haloperidol (1 mg kg^{-1} i.p., 60 min before death) and by (+)-, but not by (−)-butaclamol (5 mg kg^{-1}, 30 min before death).

In-vitro binding experiments. Scatchard analysis of specific in-vitro [^{3}H]spiperone binding (as defined by (−)-sulpiride or (+)-butaclamol) revealed a small number of specific saturable binding sites in membrane preparations from the superior colliculus (the number of sites in the superior colliculus were 24 and 29% respectively of those in striatal tissue) (Table 1; Fig. 3). The K_D for [^{3}H]spiperone binding was approximately

Table 1. The number of binding sites (B_{max}) and the equilibrium dissociation constant (K_D) for specific [^{3}H]spiperone (0·1–6·0 nM) and [^{3}H]*N*,n-propylnorapomorphine (NPA) (0·05–3·0 nM) binding to striatal and superior colliculus tissue preparations from rat brain.

Brain area and displacing agent	[^{3}H]Spiperone B_{max} (pmol g^{-1})	[^{3}H]Spiperone K_D (nM)	[^{3}H]NPA B_{max} (pmol g^{-1})	[^{3}H]NPA K_D (nM)
Striatum				
(−)-Sulpiride	21·2 ± 1·6	0·19 ± 0·03	—	—
(+)-Butaclamol	29·2 ± 2·3	0·16 ± 0·02	11·6 ± 1·3	0·82 ± 0·09
(±)-ADTN	—	—	17·1 ± 1·5	1·02 ± 0·04
Superior colliculus				
(−)-Sulpiride	5·0 ± 0·4	1·35 ± 0·29	—	—
(+)-Butaclamol	8·5 ± 0·9	1·04 ± 0·4	No specific binding observed	
(±)-ADTN	—	—	1·20 ± 0·30	1·48 ± 0·30

Values are expressed as the mean (±1 s.e.m.) of a minimum of 6 independent determinations. In each individual experiment pooled tissue from 12 animals was examined in triplicate at each of at least 6 ligand concentrations. Data was subjected to Scatchard analysis followed by linear regression analysis to obtain each estimate of B_{max} and K_D. Specific binding represented 25–65% of total [^{3}H]NPA binding and 35–75% of total [^{3}H]spiperone binding depending on the tissue and the concentration of ligand. The correlation coefficient for Scatchard analysis in each experiment was not less than 0·90.

10-fold higher than that detected in striatal preparations (Table 1).

Specific in-vitro [^{3}H]NPA binding (as defined by (±)-ADTN) to tissue preparations from the superior colliculus showed that B_{max} to be only 7% of that observed in the striatum (Table 1; Fig. 4). No specific binding could be demonstrated using (+)-butaclamol. K_D values for specific [^{3}H]NPA binding to the superior colliculus were aproximately the same as those found in the striatum (Table 1).

Histology. Histological examination of brains following removal of the superior colliculus showed the area taken to extend from A 2·0 to P 1·0 (Konig & Klippel 1963). In all cases the superficial and intermediate layers were removed and deeper layers were removed in some cases. The only other damage of note was the removal in some cases of very small areas of the overlying cortex.

Discussion

The in-vivo accumulation of radioactivity derived from [^{3}H]spiperone or [^{3}H]NPA in the superior colliculus and its displacement by systemic administration of dopamine-active drugs in a stereoselective manner, suggests the presence of dopamine receptors in this structure. It is known that [^{3}H]spiperone can also label 5-HT receptors and, possibly, noradrenergic sites. However, displacement of this ligand from the superior colliculus by sulpiride, which is highly selective for dopamine receptors, makes this unlikely. This was confirmed by the failure of the 5-HT antagonist, cinanserin, and of the adrenergic agents, propranolol and prazosin, to displace in-vivo binding of [^{3}H]spiperone from the superior colliculus. An identical profile of drug displacement was apparent in the striatum. However, in the frontal cortex (data not shown), where [^{3}H]spiperone predominantly labels 5-HT receptors, administration of cinanserin caused displacement of the ligand. The specificity for displacement of in-vivo binding of [^{3}H]spiperone by a range of dopamine antagonist drugs argues against the ligand binding to saturable non-specific sites. Furthermore, in-vivo [^{3}H]NPA also accumulated in the superior colliculus and could be displaced by dopamine agonists and some antagonists, suggesting that the ligand also identified dopamine receptor sites in this region. There is no evidence to suggest that [^{3}H]NPA labels receptors other than dopamine or that non-specific saturable sites exist.

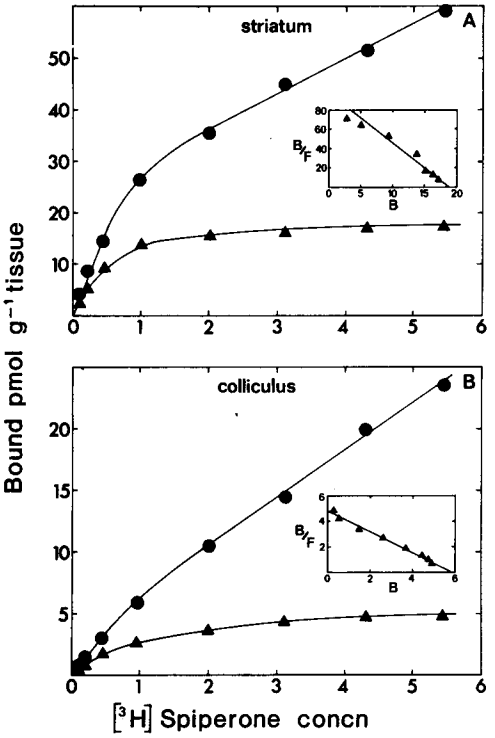

FIG. 3. Total and specific [^{3}H]spiperone binding, together with Scatchard analysis, to rat striatal (A) and superior colliculus (B) membrane preparations. Membranes were prepared in Tris-HCl (50 mM; pH 7·6) and finally re-homogenized in Tris-Na$^+$. Aliquots were incubated with increasing concentrations of [^{3}H]spiperone (0·1–6·0 nM) at 37 °C for 10 min. Specific binding was defined by incorporation of (−)-sulpiride (10^{-5} M). ● Total binding. ▲ Specific binding. Data represent the mean of triplicate determinations from a single typical experiment. At least six independent experiments were performed to provide the data in Table 1. The inset represents Scatchard transformation of the data, followed by linear regression to provide estimates of binding parameters.

	B_{max} (pmol g^{-1})	K_D (nM)	r
Striatum	18·6	0·19	0·94
Superior colliculus	5·7	1·20	0·95

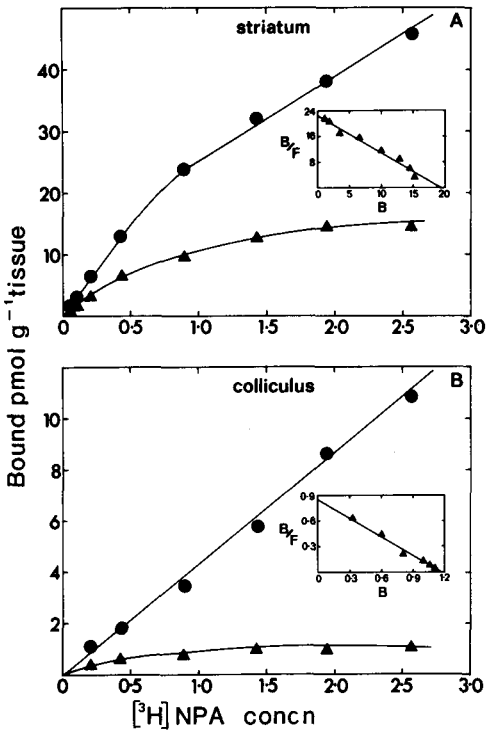

FIG. 4. Total and specific [³H]NPA binding, together with Scatchard analysis, to rat striatal (A) and superior colliculus (B) membrane preparations. Membranes were prepared in Tris-EDTA (pH 7·8). Aliquots were incubated with increasing concentrations of [³H]spiperone (0·05–3·0 nM) at 37 °C for 10 min. Specific binding was defined by incorporation of (±)-ADTN (10^{-6} M). ● Total binding. ▲ Specific binding. Data represent the mean of triplicate determinations from a single typical experiment. At least six independent experiments were performed to provide the data in Table 1. The inset represents Scatchard transformation of the data, followed by linear regression to provide estimates of binding parameters.

	B_{max} (pmol g^{-1})	K_D (nM)	r
Striatum	19·3	0·90	0·94
Superior colliculus	1·16	1·38	0·90

The results from this study were unexpected since we are unaware of any evidence for dopamine innervation of this region, but it is uncertain whether appropriate histological investigation has centred on the superior colliculus. Measurement of the concentration of dopamine in the superior colliculus has revealed only low levels (Versteeg et al 1976). However, no measurements of metabolite levels or more importantly of turnover have been undertaken. Interestingly, in an autoradiographic study of the localization of [³H]spiperone in rat brain, labelling was demonstrated in the superior colliculus but was dismissed as being associated with 5-HT receptors on the grounds that dopamine innervation of this area was unknown (Klemm et al 1979).

Using in-vitro ligand binding techniques, we could confirm the presence of sites labelled by [³H]spiperone, although the number was small compared with striatum and their affinity low. Again, definition of these sites using sulpiride suggests they are dopamine receptors rather than 5-HT or noradrenaline sites. However, the number of sites identified by (+)-butaclamol was larger than found with sulpiride, suggesting some binding of [³H]spiperone to 5-HT sites in-vitro. Using [³H]NPA in-vitro, we found identification of sites was difficult and inconsistent. The number of sites present was extremely small and could only be identified by definition with ADTN but not (+)-butaclamol. However, this does not negate the existence of dopamine receptors in this region for the density of the dopamine receptor population may be too low to detect using this in-vitro technique. There is precedent for such a situation in that while dopamine receptors have been shown to exist in the hippocampus using in-vivo binding techniques, they cannot be detected by in-vitro methods (Bischoff et al 1979, 1980; Scatton, personal communication). Similarly, while dopaminergic innervation of frontal cortex is known to exist from anatomical studies, dopamine receptors cannot be readily demonstrated using in-vitro ligand binding techniques.

In conclusion, we suggest that the superior colliculus contains dopamine receptors and may receive dopaminergic innervation from a presently unknown source. *Precedence for the existence of a descending dopamine* tract arising from substantia nigra comes from the discovery of an uncrossed pathway passing to spinal cord (Commissiong & Neff 1979). Such dopamine receptors in the superior colliculus may be involved in the control of motor behaviour. Thus, in a recent study, Starr & Summerhayes (1982) were unable to show any behavioural effect of a focal unilateral injection of apomorphine into the superior colliculus in normal rats, but in animals with a prior 6-OHDA lesion of the medial forebrain bundle the same treatment resulted in tight contralateral rotation, which was blocked by haloperidol.

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Central pressor activity of cimetidine in spontaneously hypertensive rats

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The systemic blood pressure effect of cimetidine given intracerebroventricularly (i.c.v.) in anaesthetized spontaneously hypertensive (SH) rats has been investigated. Cimetidine (250 μg i.c.v.) caused a gradual long lasting rise in mean in mean arterial blood pressure with maximum of 31·6 ± 4·5 mm Hg. Chemical degeneration of catecholaminergic neurons with 6-OHDA treatment, central administration of phentolamine and prazosin, and the bilaterial adrenalectomy significantly inhibited the pressor response of cimetidine, while propranolol (i.c.v.) had no effect. From these results it appears that the hypertensive response of cimetidine is mediated by central catecholaminergic pathways and is due to an increase in efferent sympathetic outflow and release of catecholamine from the adrenal medulla.

Central administration of H_2-receptor antagonists has been shown to increase blood pressure in anaesthetized animals (Finch & Hicks 1976; Karppanen et al 1977: Paakkari et al 1982), the mechanism of which is unclear. It is probably not due to H_2-receptor blockade, since activation of central histamine receptors causes hypertension (Finch & Hicks 1976; Finch et al 1978; Karppanen et al 1977), however the in-vivo experiments of Albinus & Sewing (1974) and Brimblecombe et al (1975) have shown that the peripheral effects of burimamide and metiamide were not directly related to H_2-receptor blockade, but were catecholamine-dependent. It has also been proposed that central administration of metiamide increases blood pressure through a mechanism involving a GABA-receptor antagonism (Antonaccio et al 1981). Studies in our laboratory showed that in anaesthetized spontaneously hypertensive (SH) rats, central administration of the H_2-receptor antagonist cimetidine, caused a sustained rise in perfusion pressure of autoperfused hindquarters (Dohadwalla & Dadkar 1981). The present study was undertaken to elucidate the mechanism responsible for vasoconstrictor action of centrally administered cimetidine in SH rats.

Materials and methods

Male SH rats (230–250 g) the strain developed by Okamoto & Aoki (1963) were used. Permanent cannulation of lateral cerebroventricles was performed stereotaxically on pentobarbitone sodium anaesthetized animals. The skull was exposed and a small hole was made through the parietal bone with the tip of 20 gauge needle, at the co-ordinate of L 1–1·5 mm, P 1·0 mm with respect to bregma. The polyethylene cannula was inserted into the cerebral ventricle, to the depth of 4·5 mm below the outer surface and fixed to the skull with dental acrylic which also enveloped a small stainless steel screw. These rats were allowed to rest for

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