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Brain Research, 194 (1980) 403–418

PROPERTIES OF DOPAMINE AGONIST AND ANTAGONIST BINDING SITES IN MAMMALIAN RETINA

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(Accepted January 24th, 1980)

Key words: retina — dopamine receptors — ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) — spiroperidol (spiperone) — striatum

SUMMARY

Retinal homogenates of calf, rat, rabbit and *Cebus appella* and *Macaca mulata* monkeys were found to contain stereospecific binding sites for the dopamine antagonist [^{3}H]spiroperidol. In further studies with calf and rat retina, stereospecific binding sites were also found for the dopamine agonist [^{3}H]ADTN (2-amino-6,7,-dihydroxy-1,2,3,4-tetrahydronaphthalene). The [^{3}H]spiroperidol binding sites in calf retina were pharmacologically similar to the dopaminergic spiroperidol binding sites previously demonstrated to be present in striatum. However, calf and rabbit retina contained less than 1/10 the concentration of [^{3}H]spiroperidol binding sites found in striatum. Saturation studies and Scatchard analyses showed a single class of [^{3}H]spiroperidol binding sites with K_d (apparent dissociation constant) = 0.3 and 0.2 nM and B_{max} (binding site number) = 38 and 24 fmol/mg protein in calf retina and rabbit retina respectively. Rates of [^{3}H]spiroperidol association and dissociation were also evaluated in calf retina. Drug specificity for [^{3}H]ADTN binding in calf retina resembled that previously reported for striatal [^{3}H]ADTN binding and thus differed from retinal [^{3}H]spiroperidol binding. Calf retinal [^{3}H]ADTN binding sites had a K_d = 9 nM and B_{max} = 113 ± 12 fmol/mg protein. Thus, the total number of [^{3}H]ADTN sites in retina was at least twice that of [^{3}H]spiroperidol sites. Guanine nucleotides (GTP and Gpp (NH)p) but not ATP reduced the affinity of the dopamine agonist ADTN for [^{3}H]spiroperidol binding, and also reduced the specific binding of [^{3}H]ADTN itself up to a maximal value of about 50% of control binding. Saturation studies of calf retinal [^{3}H]ADTN binding confirmed that Gpp(NH)p-displaceable sites were a discrete saturable subset of stereospecific [^{3}H]ADTN sites with K_d = 9 nM and B_{max} = 50 ± 6 fmol/mg protein. The Gpp(NH)p insensitive sites had a K_d = 9 nM and B_{max} = 63 ± 7 fmol/mg protein. It is proposed that although [^{3}H]ADTN sites

differ pharmacologically from [^{3}H]spiroperidol sites, since [^{3}H]spiroperidol sites are guanine nucleotide-sensitive and similar in number to the guanine nucleotide-sensitive class of [^{3}H]ADTN sites, they may possibly be related to these sites as well as to adenylate cyclase. In addition, retina contains guanine nucleotide-insensitive [^{3}H]-ADTN sites, possibly presynaptic and probably not coupled to adenylate cyclase.

INTRODUCTION

Dopamine, the principal catecholamine of the mammalian retina[24], and dopamine agonists stimulate retinal adenylate cyclase[1,19], a stimulation blocked by antipsychotic drugs known to be dopamine antagonists in other systems[2]. Dopamine receptors coupled to adenylate cyclase in retina are pharmacologically similar to those in striatum, but may be distinguished from cortical types of dopamine receptors associated with adenylate cyclase[16,17]. In other regions of the central nervous system dopamine receptors have been assessed not only with use of adenylate cyclase but also by means of direct binding of radioactive dopamine agonists and antagonists to receptor sites. However, dopamine antagonist binding sites, measured by [^{3}H]spiroperidol or [^{3}H]haloperidol, appear from extensive characterization studies in striatum[5,6,9,12,26,27] to differ pharmacologically from the striatal or retinal as well as from other types of dopamine receptors associated with adenylate cyclase[16,17,27]. Dopamine agonist binding sites measured by [^{3}H] apomorphine[8,23,25], [^{3}H]dopamine[5] or [^{3}H]ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene)[7,23] differ pharmacologically from antagonist binding sites in striatum and may in fact resemble more closely dopamine receptors associated with adenylate cyclase. Classes of dopamine receptors in striatum have also been differentiated on the basis of sensitivity to guanine nucleotides[7,8,23,28], with the guanine nucleotide-sensitive sites possibly selectively coupled to adenylate cyclase.

We report here the presence of stereospecific, saturable and high affinity binding sites for [^{3}H]spiroperidol and [^{3}H]ADTN in the retina of several mammalian species, as well as pharmacological characterization of these sites and examination of these sites for guanine-nucleotide sensitivity. It is concluded from these studies that the retina contains [^{3}H]spiroperidol binding sites exclusively dopaminergic in nature and a significantly greater number of [^{3}H]ADTN binding sites, with the latter capable of being subdivided into two distinct classes of guanine nucleotide-sensitive and guanine nucleotide-insensitive sites. Some of these findings have been reported previously in preliminary form[20].

METHODS

Fresh calf eyes were removed as rapidly as possible after the animals were killed and the eyes kept on ice for a time period of about 1–2 h prior to dissection of the retinas. Retinas were also obtained from New Zealand white rabbits (5 months of age), rhesus (*Macaca mulata*) and *Cebus appella* monkeys (less than 5 years old) and

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TABLE III

[^{3}H]ADTN binding site in calf and rat retina: stereospecificity and influence of guanine nucleotides

Values represent means ± S.E.M. of 7 experiments (calf) or 3 experiments (rat). [^{3}H]ADTN concentration was 4 nM. Other details were as described in Methods.

Species and additions to binding assay	*fmol [^{3}H]ADTN displaced/mg protein*
Calf (total binding = 111 + 3 fmol/mg protein):	
(—)-Butaclamol (10 μM)	3 ± 5
(+)-Butaclamol (10 μM)	58 ± 2
Gpp(NH)p (50 μM)	28 ± 2
GTP (50 μM)	28 ± 3
GTP (50 μM) + (+)-Butaclamol (10 μM)	52 ± 4
GTP (100 μM)	32 ± 4
ATP (50 μM)	4 ± 4
ATP (100 μM)	5 ± 6
Rat (total binding = 94 + 5 fmol/mg protein):	
(—)-Butaclamol (1 μM)	2 ± 2
(+)-Butaclamol (1 μM)	41 ± 1
GTP (50 μM)	30 ± 3
GTP (100 μM)	20 ± 2

unlabeled ligand was also rapid, with only a slight deviation from linearity evident after 10 min when ln[receptor concentration bound] is plotted vs time (Fig. 3). Thus dissociation apparently involved a single class of receptors, with a $t_{1/2}$ of dissociation of 4.7 min and a $k_{-1} = 0.146$ min^{-1}. The dissociation constant (K_d) calculated from the ratio of k_{-1} to k_1 is 0.36 nM, a value similar to that determined in the saturation experiments shown in Fig. 1.

[^{3}H]LSD binding in retina

In striatum [^{3}H]LSD has been reported to bind to both dopaminergic and serotonergic receptor classes[4]. There exists essentially no evidence for serotonergic transmission in retina, and low amounts of N-acetylserotonin but not serotonin itself, have been found in retina[3]. Thus, it seemed possible that [^{3}H]LSD might be useful in labeling dopamine receptors in retina. However, at a ligand concentration of 0.9 nM only 17 fmol [^{3}H]LSD/mg protein bound to calf retinal preparations and there was no significant displacement of bound radioactivity by dopamine or serotonin agonists or antagonists (data not shown). In contrast, striatum assayed under the same conditions bound 42 fmol [^{3}H]LSD/mg protein, of which 14 fmol/mg and 12 fmol/mg were displaced by 1 μM serotonin and 10 μM dopamine respectively, 27 fmol/mg were displaced by 1 μM (+)-butaclamol, and 26 fmol/mg were displaced by a combination of 1 μM serotonin plus 10 μM dopamine.

[^{3}H]ADTN binding in retina

Since [^{3}H]ADTN has proven useful as a radioligand for assessing dopamine agonist receptor sites in striatum[7,23], this ligand was also investigated in retina. With calf and rat retinal preparations and with [^{3}H]ADTN present at 3–6 nM, total binding