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α-ADRENERGIC RECEPTORS IN THE RAT SUPERIOR CERVICAL GANGLION

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Abstract—α-Adrenergic receptor sites on membranes from rat superior cervical ganglia were measured by the specific binding of [^{3}H]dihydroergocryptine, an α-adrenergic antagonist. The binding was specific, saturable, reversible, rapid, and stereoselective. Specific binding was displaced by α-adrenergic agonists in the expected order of potency and by α-adrenergic antagonists, but only weakly by a β-adrenergic agonist and antagonist. Decentralization of the ganglia by severing the preganglionic cholinergic nerve supply resulted in a marked decrease in the number of α-adrenergic receptors. The data suggest that about one-half the α-adrenergic receptors are located on the preganglionic cholinergic axon terminals and one-half on ganglionic cell membranes.

α-Adrenergic receptors mediate a negative feedback mechanism inhibiting the firing of central noradrenergic neurons [1] and the release of norepinephrine (NE) from brain slices [2, 3] and the peripheral sympathetic nervous system [4, 5]. The α-adrenergic receptors postulated to be responsible for the inhibition of neuronal transmission are pre- as contrasted with post-synaptic receptors [5–11]. In the rat, superior cervical ganglion stimulation of pre-synaptic nerves leads to neuronal transmission through the ganglion and to the release of NE at the endings of ganglionic noradrenergic neurons. It is hypothesized that an increase in NE concentration in the synapse activates pre-synaptic α-adrenergic receptors which inhibit further release of NE by an unknown mechanism possibly involving diminished availability of cytoplasmic calcium necessary to the exocytotic process.

The rat superior cervical ganglion is devoid of post-synaptic adrenergic end-organs. It has only preganglionic cholinergic nerve endings, glia, ganglionic noradrenergic neurons, and a few interneurons. The ganglionic neurons and their axons, which terminate in various tissues and blood vessels in the head and neck, constitute the noradrenergic sympathetic innervation to these areas. The present study was undertaken to establish the presence of α-adrenergic receptors in the ganglion, to measure their number, and partially to characterize them.

MATERIALS AND METHODS

The binding of the α-adrenergic antagonist [^{3}H]dihydroergocryptine to membranes of the superior cervical ganglia was used to measure the α-adrenergic receptor [12, 13]. Male Sprague–Dawley rats weighing 150–160 g were killed by a blow to the head and the superior cervical ganglia were removed, decapsulated, and put in NaCl (0.85%) buffered with Na^+–K^+ phosphate (0.006 M) to pH 7.1 at 4°, a temperature maintained throughout the membrane preparation. For studies in which the ganglion was decentralized, rats were anesthetized with sodium nembutal (60 mg/kg) and the preganglionic nerve fibers to the right superior cervical ganglion were cut 7 days before death. The development of ptosis on the operated side established the completeness of the lesion. The unoperated contralateral ganglion served as the control tissue.

About 100 ganglia were rinsed in Tris buffer (0.05 M, pH 7.4) and homogenized in 2 ml of buffer using a Polytron homogenizer for three 15-sec intervals. The homogenate was centrifuged at 12,000 *g* for 10 min, and the pellet was resuspended for the binding assay with a 15-sec homogenization in Tris buffer. The protein concentration of the membrane suspension was measured by the method of Lowry *et al.* [14].

[^{3}H]Dihydroergocryptine (14.6 nM unless otherwise specified) and the membrane preparation (0.3 to 0.4 mg protein/incubation in a total volume of 150 μl), with or without neurotransmitters or drugs as indicated, were incubated at 25° for 22 min. Incubations were terminated by rapid filtration under vacuum through Whatman GF/C filters and washed four times with 7 ml of Tris buffer. The washes decreased nonspecific binding without lowering specific binding, defined as that part of the total binding which was displaced by 1×10^{-4} M phentolamine, a concentration of the α-adrenergic antagonist which maximally occupies α-adrenergic receptor sites. Tritium retained on the filters was assayed by liquid scintillation spectrometry using Aquasol as scintillation fluid. Specific binding represented about 60 per cent of the total bound ^{3}H, about 1800 cpm/incubation in a typical experiment. Incubations done in triplicate agreed within ± 10 per cent.

Compounds used were Tris–HCl, *l*-epinephrine, *l*-norepinephrine, *dl*-propranolol and *l*-isoproterenol (Sigma Chemical Co., St. Louis, MO), *d*-norepinephrine (Adams Chemical Co., Cleveland, OH), and [^{3}H]dihydroergocryptine (24.1 Ci/m-mole) (New England Nuclear, Boston, MA). Phentolamine was a gift from Ciba Pharmaceutical Co. (Summit, NJ), phenoxybenzamine from Smith, Kline & French Laboratories (Philadelphia, PA), clonidine from Boehringer Ingelheim Ltd. (Elmsford, NY), dihydroergocryptine from Sandoz Pharmaceuticals (East Hanover, NJ), and piperoxane from Rhône–Poulenc Industries (Vitry-sur-Seine, France).

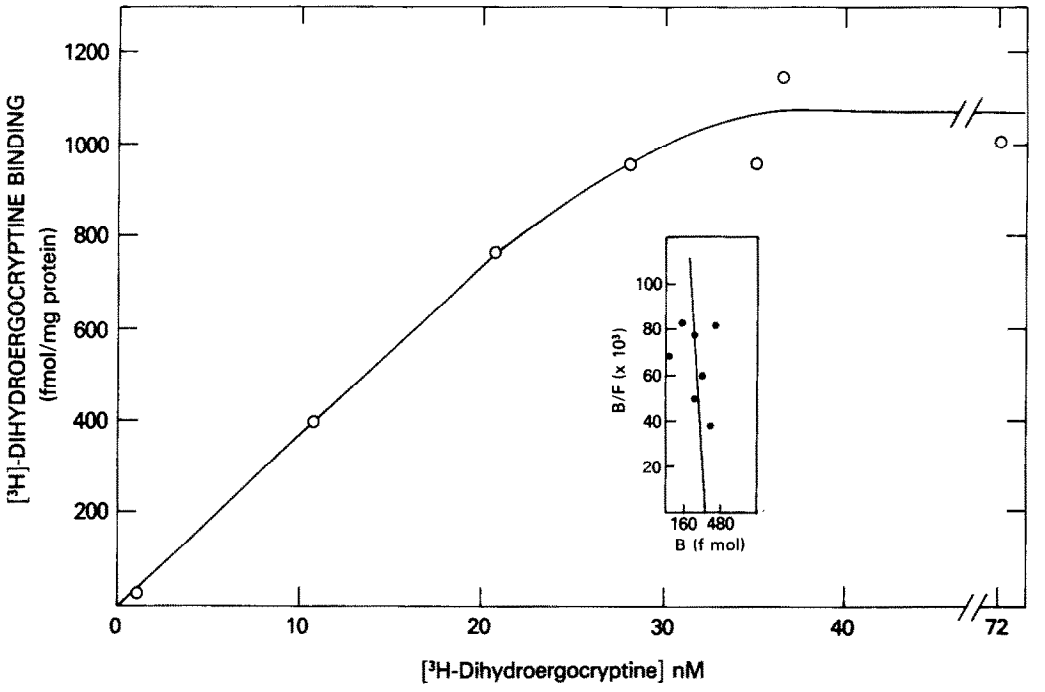

Fig. 1. Saturability of specific binding of [^{3}H]dihydroergocryptine to rat superior cervical ganglion membranes as a function of [^{3}H]dihydroergocryptine concentration. In a typical experiment, representative of four, ganglionic membranes were incubated with the indicated concentrations of [^{3}H]dihydroergocryptine as described in Materials and Methods. Each value is the mean of triplicate measurements agreeing within ± 10 per cent. The number of binding sites was computed from the intercept of a Scatchard plot of the [^{3}H]dihydroergocryptine binding data with the abcissa and from the protein concentration of the incubation, 0.33 mg.

RESULTS

The specific binding of [^{3}H]dihydroergocryptine to sites on superior cervical ganglion membranes was saturable, reaching half-maximal saturation at 14.6 nM [^{3}H]dihydroergocryptine (Fig. 1). A Scatchard plot (see Fig. 1 insert) of the specific binding was consistent with a single class of binding sites with a density at saturation calculated to be 1074 fmoles/mg of protein. The K_D was 14.6 nM.

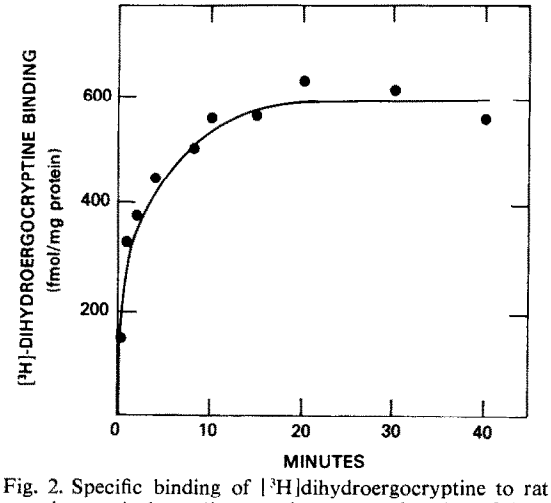

Fig. 2. Specific binding of [^{3}H]dihydroergocryptine to rat superior cervical ganglion membranes as a function of time. [^{3}H]Dihydroergocryptine (14.6 nM) was incubated with ganglion membranes for the lengths of time indicated. Each value is a mean of triplicate measurements from two experiments.

The specific binding of [^{3}H]dihydroergocryptine was rapid, reaching half-maximal binding at 1 min and equilibrium at 17 min (Fig. 2). Specific binding was reversible with a half-life of dissociation of 3.7 min (Fig. 3).

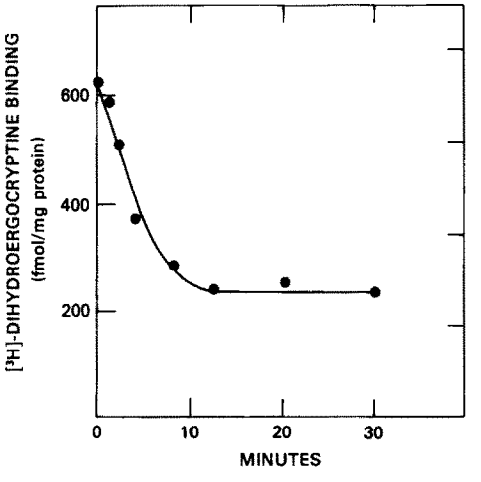

Fig. 3. Reversibility of [^{3}H]dihydroergocryptine binding to rat superior cervical ganglion membranes as a function of time. Membranes were incubated with [^{3}H]dihydroergocryptine (14.6 nM) for 22 min at 25°. At $t = 0$, 7 ml of Tris buffer (pH 7.4, 0.05 M) containing phentolamine ($1 < 10^{-4}$ M) was added. At subsequent times indicated binding was measured. Maximum binding is the amount of [^{3}H]dihydroergocryptine bound prior to phentolamine addition. Each value is the mean of triplicate measurements from two experiments.

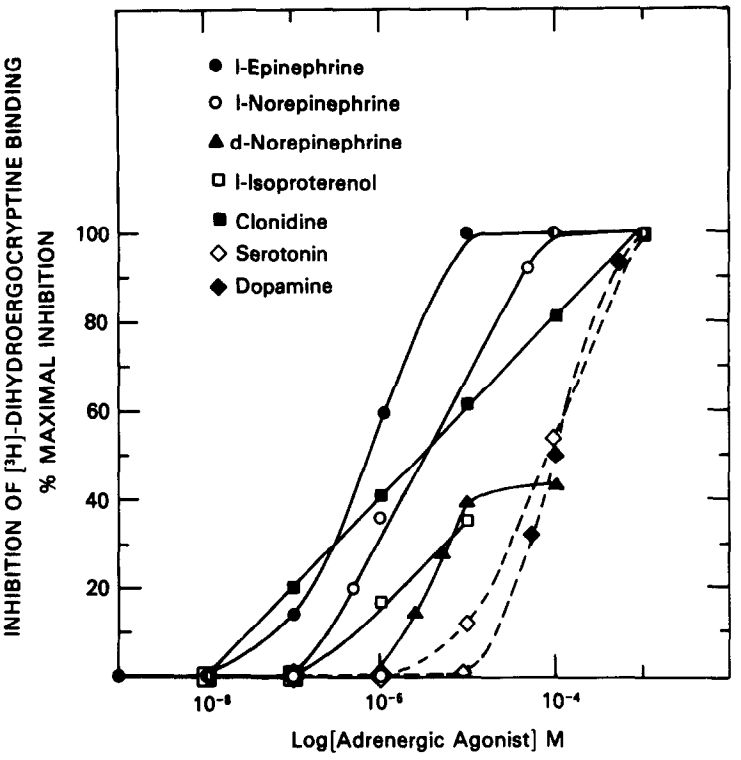

Fig. 4. Effect of adrenergic agonists and neurotransmitters on the specific binding of [^{3}H]dihydroergocryptine to rat superior cervical ganglion membranes. Each experiment was repeated two to three times with each point measured by triplicate assays agreeing within ± 10 per cent. Each point shown is the mean of two to three experiments. Ganglion membranes were incubated with [^{3}H]dihydroergocryptine (14.6 nM) and the agonist or neurotransmitter. One hundred per cent inhibition is inhibition of binding by phentolamine (1×10^{-4} M).

l-Epinephrine was four times more potent than *l*-norepinephrine or clonidine in inhibiting the specific binding of [^{3}H]dihydroergocryptine. *d*-Norepinephrine, a stereoisomer of *l*-norepinephrine, was much less potent than *l*-norepinephrine, inhibiting binding by only 44 per cent at 10^{-4} M. *l*-Isoproterenol inhibited by only 35 per cent at 1×10^{-5} M (Fig. 4, Table 1).

Table 1. Inhibition of [^{3}H]dihydroergocryptine binding by neurotransmitters and drugs

Compound	K * (M)
α-Agonists and other neurotransmitters	
l-Epinephrine	4×10^{-7}
l-Norepinephrine	1.6×10^{-6}
d-Norepinephrine	†
l-Isoproterenol	†
Clonidine	1.5×10^{-6}
Dopamine	6×10^{-5}
Serotonin	4.5×10^{-5}
α-Antagonists	
Phentolamine	3.5×10^{-7}
dl-Propranolol	†
Piperoxane	5×10^{-7}
Dihydroergocryptine	2×10^{-8}

* The value of the K_I is calculated from the concentration required to produce 50 per cent inhibition (IC_{50}) of [^{3}H]dihydroergocryptine specific binding at 14.6 nM [^{3}H]-dihydroergocryptine, the K_D for [^{3}H]dihydroergocryptine as measured in saturation experiments (Fig. 1) after Cheng and Prusoff [15].

† Binding is inhibited by < 50 per cent at 1×10^{-5} M (*l*-isoproterenol and *dl*-propranolol) and 1×10^{-4} M (*d*-norepinephrine), the highest concentrations tested.

Phentolamine and piperoxane inhibited [^{3}H]dihydroergocryptine specific binding with about the same potency as *l*-epinephrine, whereas dihydroergocryptine was 18 times more potent. *dl*-Propranolol inhibited by only 7 per cent at 1×10^{-5} M (Fig. 5, Table 1).

Except where indicated (Table 1), the maximum inhibition of [^{3}H]dihydroergocryptine binding by all the agents was of the same magnitude as that of phentolamine (1×10^{-4} M).

The binding of [^{3}H]dihydroergocryptine to decentralized ganglia was saturable, reaching half-saturation at 16.6 nM (Fig. 6, Table 2). A Scatchard plot (see Fig. 6 insert) of the specific binding showed a single class of binding sites with a maximal density at saturation of 535 fmoles/mg of protein. The calculated K_D was 16.6 nM.

DISCUSSION

[^{3}H]Dihydroergocryptine binding has been used as a measure of α-adrenergic receptors in rabbit uterus [16], bovine and rat brain [17–19], rat parotid cells [20], and human platelets [13, 21, 22].

The binding of [^{3}H]dihydroergocryptine to sites on membranes from rat superior cervical ganglia is specific, saturable, rapid, reversible and stereoselective.

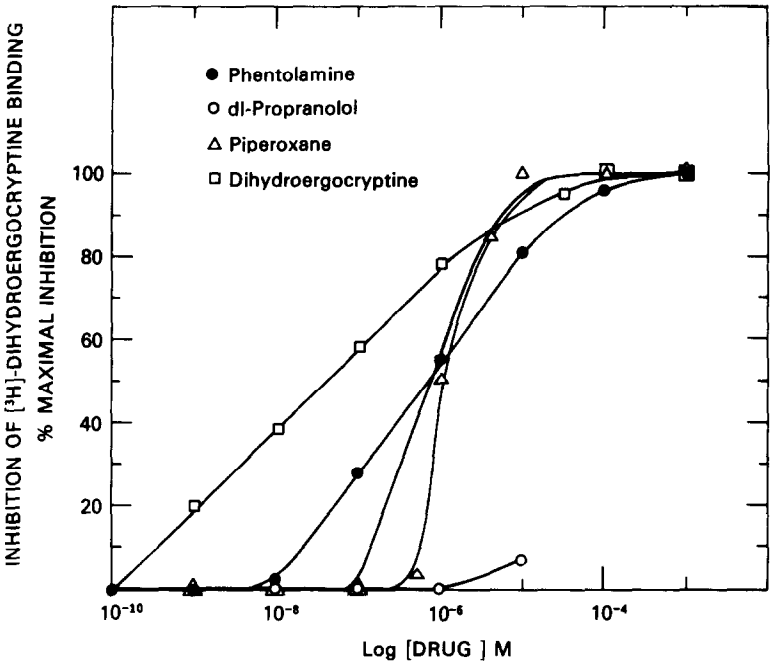

Fig. 5. Effects of drugs on the specific binding of [^{3}H]dihydroergocryptine to rat superior cervical ganglion membranes. Each experiment was repeated two to three times with each point the mean of triplicate assays agreeing within ± 10 per cent. Ganglion membranes were incubated with [^{3}H]dihydroergocryptine (14.6 nM) with and without the indicated drug. One hundred per cent inhibition is inhibition of binding by phentolamine (1×10^{-4} M).

Table 2. [^{3}H]Dihydroergocryptine binding to superior cervical ganglion membranes*

	[nM] at half-maximal saturation, K_D	Amount bound maximally (fmoles/mg protein)
Intact	14.6 ± 0.3	1108 ± 49
Decentralized	16.6 ± 0.3	540 ± 33

* Data are derived from saturation experiments: four with intact ganglia and three with denervated ganglia. Values are means ± S.E.M.

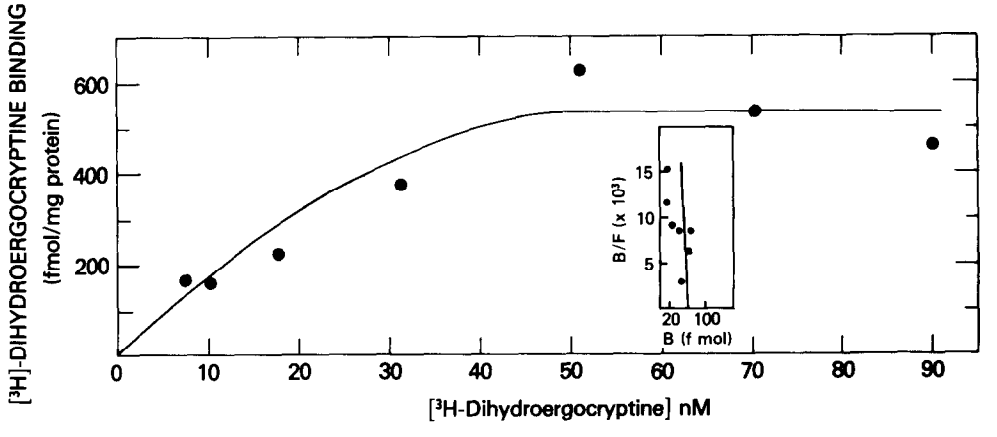

Fig. 6. Saturability of specific binding of [^{3}H]dihydroergocryptine to decentralized rat superior cervical ganglion membranes. In a typical experiment, representative of three, ganglia were decentralized and membranes incubated with indicated concentrations of [^{3}H]dihydroergocryptine as described in Materials and Methods. Each value is the mean of triplicate measurements agreeing within ± 10 per cent. The number of binding sites was computed from the intercept of a Scatchard plot of the [^{3}H]dihydroergocryptine binding data with the abscissa and from the protein concentration of the incubation, 0.11 mg.

Adrenergic agonists *l*-epinephrine, *l*-norepinephrine, clonidine and *l*-isoproterenol displace [^{3}H]dihydroergocryptine binding in the order of potency expected of α- as contrasted with β-adrenergic agonists [23–25], *l*-epinephrine being four times as potent as *l*-norepinephrine and *l*-isoproterenol acting weakly. *d*-Norepinephrine, a biologically inactive stereoisomer of *l*-norepinephrine, is a very weak inhibitor of binding.

The α-adrenergic antagonists, dihydroergocryptine, phentolamine and piperoxane, are potent inhibitors of [^{3}H]dihydroergocryptine binding, dihydroergocryptine being 20 times as potent as *l*-epinephrine, and phentolamine and piperoxane being approximately equipotent with *l*-epinephrine. *dl*-Propranolol, a β-adrenergic antagonist, is almost inactive in inhibiting binding. Antagonist inhibition of binding is also that expected of α-, not β-, adrenergic drugs.

In rat and bovine brain [^{3}H]dihydroergocryptine binds specifically to different kinds of α-adrenergic receptors [18, 19, 26] characterized in peripheral tissues as α_1- and α_2-adrenergic receptors [27]. Further studies are necessary to distinguish the type(s) of α-adrenergic receptor present in the rat superior cervical ganglion because our data do not permit this discrimination.

Membranes from decentralized ganglia show about 50 per cent as much [^{3}H]dihydroergocryptine bound at half-saturation as do membranes from unoperated ganglia (Figs. 1 and 6, Table 2). Because the affinities for dihydroergocryptine are about the same in membranes from decentralized and from unoperated ganglia (Table 2), the membranes from decentralized ganglia have only half the number of α-adrenergic receptor sites. Decentralization involves severing and subsequent degeneration of cholinergic fibers to the ganglion without significant atrophy of noradrenergic neurons or other cells present in the ganglion [28]. It is likely, therefore, that the α-adrenergic receptors which disappear with decentralization are on cholinergic axon terminals. Evidence for physiological actions of α-adrenergic receptors on preganglionic cholinergic axons comes from several sources. *l*-Norepinephrine has been shown to inhibit the per pulse release of acetylcholine in parasympathetic nerve terminals [29, 30] *in situ* and *in vitro* in the gut of several species. *l*-Epinephrine and *l*-norepinephrine reduce ganglionic transmission in the gut and in rat superior cervical ganglia [31, 32], and *l*-norepinephrine hyperpolarizes superfused rat superior cervical ganglia through an action on α-adrenergic receptors [11]. It seems likely, then, that *l*-norepinephrine secreted by the densely arborized noradrenergic ganglionic neurons of the rat superior cervical ganglion [33] can occupy α-adrenergic receptors on preganglionic cholinergic axonal terminals. Under physiological conditions, *l*-norepinephrine occupying these α-adrenergic receptors may inhibit the release of acetylcholine by the preganglionic neurons, decreasing transmission through the ganglion [5]. If some of the remaining half of the α-adrenergic receptors are located on axons of noradrenergic ganglionic neurons, these α-adrenergic receptors may act, in turn, to diminish further the release of norepinephrine by the ganglionic neurons. The result would be a decrease in electrical transmission through the ganglion, a decrease in the release of norepinephrine by ganglionic noradrenergic neurons and a diminution of the actions of the superior cervical ganglion on end organs and tissues.

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