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CHOLINE AS AN AGONIST: DETERMINATION OF ITS AGONISTIC POTENCY ON CHOLINERGIC RECEPTORS

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Abstract—These experiments examined the potency of choline as a cholinergic agonist at both muscarinic and nicotinic receptors in rat brain and peripheral tissues. Choline stimulated the contraction of isolated smooth muscle preparations of the stomach fundus, urinary bladder and trachea and reduced the frequency of spontaneous contractions of the right atrium at high micromolar and low millimolar concentrations. The potency of choline to elicit a biological response varied markedly among these tissues; EC_{50} values ranged between 0.41 mM in the fundus to 14.45 mM in the atrium. Choline also displaced [^{3}H]quinuclidinyl benzilate binding in a concentration-dependent manner although, again, its potency varied among different brain regions (K_i = 1.2 to 3.5 mM) and peripheral tissues (K_i = 0.28 to 3.00 mM). Choline exhibited a comparable affinity for nicotinic receptors. It stimulated catecholamine release from the vascularly perfused adrenal gland (EC_{50} = 1.3 mM) and displaced L-[^{3}H]nicotine binding to membrane preparations of brain and peripheral tissues (K_i = 0.38 to 1.17 mM). However, the concentration of choline required to bind to cholinergic receptors in most tissues was considerably higher than serum levels either in controls (8–13 μM) or following the administration of choline chloride (200 μM). These results clearly demonstrate that choline is a weak cholinergic agonist. Its potency is too low to account for the central nervous system effects produced by choline administration, although the direct activation of cholinergic receptors in several peripheral tissues may explain some of its side effects.

Choline elicits a variety of pharmacological effects in both humans and laboratory animals [1]. Under appropriate circumstances, treatments that raise circulating and tissue choline levels cause a parallel increase in acetylcholine synthesis and release [2–5] and enhance cholinergic transmission, causing functional changes in neurons and endocrine cells postsynaptic to those with elevated acetylcholine levels [6, 7]. These findings indicate that choline exerts its cholinergic effects by elevating releasable acetylcholine levels through increased precursor availability. It has long been known, however, that choline also has the ability to act as an agonist at cholinergic receptors, and so it is possible that some of the cholinomimetic effects of choline may result from a direct activation of cholinergic receptors (see Ref. 8). We have shown previously that choline slows the spontaneous contractions of the isolated atrium of the rat and guinea pig although relatively high choline concentrations (greater than 1 mM) were required to produce a measurable pharmacological response [9]. Similarly, it has been shown that choline displaces the muscarinic antagonist, [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB), from binding sites in rat brain homogenates only at millimolar concentrations [10–12]. We have also found, however, that choline stimulates contractions of the isolated guinea pig ileum at much lower concentrations (EC_{50} = 180 μM) [9]. These and earlier observations clearly indicate that, while choline does indeed stimulate muscarinic receptors, its agonistic potency is quite variable and depends upon the tissue preparation studied.

It was of interest, therefore, to investigate the agonist potency of choline at not only muscarinic but also nicotinic receptors in a variety of tissues to determine whether levels of circulating choline, either in controls or following the administration of exogenous choline, are likely to be associated with direct cholinergic receptor stimulation. The present study examined the muscarinic and nicotinic receptor potency of choline using both isolated tissue preparations and receptor binding assays to determine whether direct cholinergic receptor stimulation makes a significant contribution to the overall pharmacological effects of choline treatment and, if so, to determine the extent to which the direct cholinomimetic effects of choline are tissue specific.

MATERIALS AND METHODS

Male Sprague–Dawley rats (200–300 g; Experimental Animal Breeding and Research Center, University of Uludag Medical Faculty, Bursa, Turkey) were killed by decapitation, and the brain and various peripheral tissues were rapidly removed from each animal and placed in ice-cold saline solution. The tissues were immediately prepared either for determining the biological response to choline or for receptor binding assays.

Isolated tissue preparations. The isolated right atrium, strips of stomach fundus and urinary bladder, and spirals of trachea were suspended in a 50-ml organ bath filled with Krebs–Ringer buffer (pH 7.4) of the following composition (mM): NaCl, 118; KCl,

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4.75; $CaCl_2$, 2.54; $MgSO_4$, 1.20; KH_2PO_4, 1.19; $NaHCO_3$, 25.0; and glucose, 11.0. The solution was bubbled with 95% O_2/5% CO_2 and maintained at 37°. The tissue preparations were allowed to equilibrate for 50–60 min, and the bath fluid was changed at 10- to 15-min intervals. The fundus and urinary bladder smooth muscle strips were suspended with an initial load of 1 g, and the tracheal spirals and the right atrium were suspended under 0.5 g tension. Tension changes were recorded isometrically with a Grass recorder (Grass Polygraph, Grass Instruments, Quincy, MA). At the end of the equilibration period, cumulative concentration–response relationships were determined by adding increasing concentrations of choline at 1-min intervals. The developing tension of rat smooth muscle strips (fundus or urinary bladder and spirals of trachea) and changes in the frequency of spontaneous contractions of the isolated right atrium produced by elevated choline concentrations in the incubation medium were reported as the percentage of the maximal response to choline.

Perfusion of the adrenal gland and measurement of catecholamine release. The left adrenal gland of the rat was isolated and perfused according to a method developed by Wakade [13]. Briefly, rats were anesthetized with ether, the abdomen was opened by a mid-line incision, the left adrenal gland was exposed, and the stomach, intestine and portions of the liver were retracted and covered with saline-soaked gauze pads. A perfusion cannula (PE 50) was inserted into the distal end of the renal vein after all the branches of the adrenal vein, the renal vein and the vena cava were ligated. A small slit was made in the adrenal cortex just opposite the entrance of the vein, and the adrenal gland, along with the ligated blood vessels and the cannula, was carefully removed and perfused with Krebs–Ringer buffer at a rate of 0.5 ml/min. The perfusion medium was bubbled continuously with 95% O_2/5% CO_2 and was maintained at 37°.

After a 30-min equilibration period the perfusate was collected for 2 min and then the lowest choline concentration to be tested was added to the perfusion medium and the perfusate was again collected for 2 min. The adrenal gland was then perfused with choline-free medium for 8 min, and a higher concentration of choline was added to the perfusion medium and the perfusate was collected for 2 min. This procedure was repeated five to seven times until the choline concentration was raised to 32 mM. The catecholamine content of the perfusate was measured directly by the fluorometric method of Anton and Sayre [14] except that the alumina extraction step was omitted. A volume of 0.25 ml of the perfusate was used for the assay. The catecholamine concentration of the perfusate was calculated as noradrenaline equivalents (ng/2 min) and was expressed as percentage of the maximum release.

Preparation of tissue homogenates and receptor binding assays. Whole brain, brain regions (cerebral cortex, corpus striatum, cerebellum, hippocampus, hypothalamus, thalamus and brainstem) and upper spinal cord were first homogenized in 10 vol. of ice-cold 0.32 M sucrose using a Teflon–glass homogenizer, and the homogenates were centrifuged for 10 min at 18,000 *g* and 4°. The pellets were then re-homogenized in a sufficient volume of 50 mM sodium/potassium phosphate buffer (pH 7.4) to produce a final concentration of approximately 10 mg tissue/ml. The homogenates were either used immediately or stored in aliquots at −20° for up to 2 days.

The submaxillary and parotid glands, pancreas, right and left atria, and the ventricles were homogenized in 50 mM sodium/potassium phosphate buffer (pH 7.4; 25 mg tissue/ml) using an Ultra-Turrax tissue homogenizer (setting 7; 30 sec). The homogenates were filtered through two layers of cheesecloth and used, freshly prepared, for receptor binding assays. The adrenal medullae, superior cervical ganglia, and inferior cervical ganglia were homogenized in 600 μl sodium/potassium phosphate buffer using a glass–glass homogenizer, and the homogenates were used fresh.

The gastrointestinal tract (fundus, ileum and colon), urinary bladder and diaphragm were minced with scissors and homogenized in sodium/potassium phosphate buffer (25 mg tissue/ml) using an Ultra-Turrax homogenizer (setting 7; 60 sec). The resulting homogenates were re-homogenized with a glass–glass homogenizer, passed through two layers of cheesecloth and used, freshly prepared, for receptor binding assays.

Receptor binding assays were performed using a modification of the method of Yamamura and Snyder [15]. Muscarinic receptor binding was examined using [^{3}H]QNB (76 Ci/mmol; Amersham Corp., England). Saturable [^{3}H]QNB binding was determined by incubating 200 μl of tissue homogenate with increasing concentrations (0.1 to 6.40 nM) of the radioligand in a final volume of 300 μl of sodium/potassium phosphate buffer (pH 8.4) at 37°. The incubations were terminated after 60 min by vacuum filtration through Whatman GF/B filters. The filters were washed three times with 3 ml of ice-cold buffer and placed in a scintillation vial containing 4 ml of Aquasol-2 (New England Nuclear, Boston, MA). Radioactivity was determined at least 3 hr later in a Packard liquid scintillation counter. Saturable binding was taken as that portion of total binding not inhibited by 10 μM atropine. Nicotinic receptor binding was examined using L-[^{3}H]nicotine (78 Ci/mmol; Amersham Corp.) [16]. Saturable binding was determined by incubating 200 μl of tissue homogenate with increasing concentrations (0.1 to 64 nM) of L-[^{3}H]nicotine in a final volume of 300 μl sodium/potassium phosphate buffer at 37°. After 40 min the incubation was terminated by filtering the samples through Whatman GF/C filters, and the filters were washed three times with 3 ml of ice-cold buffer. Specific binding was determined as the total radioligand binding minus binding in the presence of 100 μM unlabeled nicotine. [^{3}H]QNB and L-[^{3}H]nicotine binding were expressed as pmol/mg tissue, and binding k_d and B_{max} were determined by Scatchard analysis of bound versus bound/free radioligand concentrations. The inhibitory potency of choline was determined by incubating a fixed concentration of [^{3}H]QNB (0.9 nM) or L-[^{3}H]nicotine (3.2 nM) with various concentrations of choline (0.01 to 100 mM).

Serum choline assay. Rats were killed by decapitation, blood was collected from the cervical wound, and serum was separated by centrifugation at 1500 *g* and 4° for 15 min. Serum choline concentrations were determined by radioenzymatic assay [17].

Materials. Reagents and supplies were purchased from the following sources: choline chloride, acetylcholine chloride, atropine sulfate, L-nicotine, physostigmine salicylate and choline kinase (Sigma Chemical Co., St. Louis, MO); [^{3}H]QNB (76 Ci/mmol), L-[^{3}H]nicotine (78 Ci/mmol) and [^{32}P]ATP (Amersham Corp., England).

RESULTS

Agonistic potency of choline in isolated smooth muscle and right atrium preparations. The sequential addition of increasing concentrations of choline to the organ bath evoked concentration-dependent contractions in isolated strips of rat fundus, urinary bladder (Fig. 1) and tracheal smooth muscle. Choline also decreased the spontaneous beating of the isolated right atrium in a concentration-dependent manner (Fig. 1). The maximum responses obtained with choline in these tissues did not differ from those produced by acetylcholine in the presence of physostigmine (20 μM) indicating that choline, like acetylcholine, is a full agonist. In all of the four tissues examined, the concentration–response curves produced by choline were shifted to the right by 10 nM atropine and were blocked completely by 100 nM atropine, indicating that the choline-induced responses were mediated by muscarinic receptors. As shown in Fig. 1 and Table 1, the agonistic potency of choline varied among the different isolated tissue preparations examined. pD_2 values for choline were essentially equivalent in the right atrium (1.84 ± 0.18) and urinary bladder (1.96 ± 0.15) but these values were considerably lower than those observed in the fundus (3.39 ± 0.10) and trachea (2.75 ± 0.15) (Table 1).

Potency of choline in stimulating catecholamine secretion from the isolated perfused rat adrenal. As shown in Fig. 2, the perfusion of the adrenal gland with increasing concentrations of choline evoked a concentration-dependent secretion of catecholamines. A significant increase above basal secretion rates was first observed at a choline concentration of 0.5 mM. Catecholamine secretion

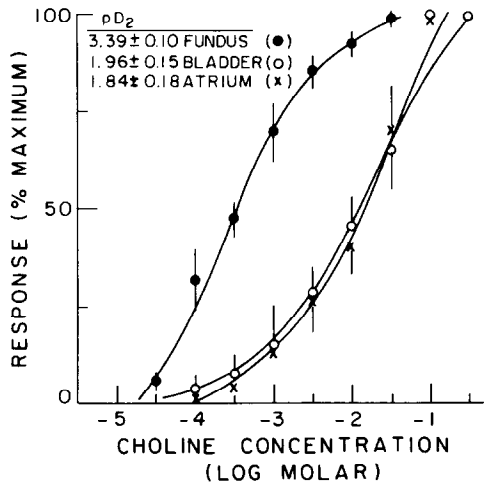

Fig. 1. Agonistic potency of choline in the isolated stomach fundus, urinary bladder and right atrium. Strips of rat stomach fundus and urinary bladder and the isolated right atrium were suspended in Krebs–Ringer buffer containing the indicated concentration of choline chloride. Contractions of fundus and bladder smooth muscle and reductions in the frequency of spontaneous atrial beats are presented as the percent maximum response to choline. The data are expressed as the mean ± SE of four to ten determinations. pD_2 values, which represent the negative logarithm of the choline concentration which produces a half-maximal response, are presented in the upper left of the figure.

increased linearly up to a maximum rate of 675 ± 75 ng/2 min (mean ± SE; N = 4) when the choline concentration was elevated to 10 nM, although further increases in the concentration of choline caused no additional increase in secretion above that obtained with 10 mM choline. The EC_{50} of choline was 2.10 ± 18 mM (N = 4 separate experiments). Acetylcholine was nearly 1000-fold more potent than choline; its EC_{50} was approximately 3 μM (mean of two experiments).

Perfusion of the adrenal gland with the nicotinic receptor antagonist mecamylamine (100 μM) completely blocked the effect of a submaximal concentration of choline (5 mM) on catecholamine secretion. A lower mecamylamine concentration (1 μM) significantly attenuated, but did not com-

Table 1. Agonistic potency of choline and acetylcholine on muscarinic receptors in rat peripheral tissues

	pD_2		EC_{50} (μM)		Relative potency	
					Choline-Tissue	ACh
Tissue	Choline	ACh	Choline	ACh	Choline-Atrium	Choline
Right atrium	1.84 ± 0.18	6.66 ± 0.24	14,450 ± 1,500	0.219 ± 0.017	1.00	66,000
Urinary bladder	1.96 ± 0.15	6.63 ± 0.05	10,960 ± 1,413	0.234 ± 0.011	1.30	47,000
Trachea	2.75 ± 0.15		1,760 ± 141		8.40	
Fundus	3.39 ± 0.10	6.59 ± 0.12	410 ± 125	0.257 ± 0.013	35.50	1,600

pD_2 values were calculated from the concentration (as -log molar)-response (as percent maximum) curves of choline or acetylcholine and represent the negative logorithm of the concentration of choline or acetylcholine which produces a 50% maximal response. The data are expressed as the mean ± SE of four to ten determinations. The tissues were incubated with acetylcholine in the presence of 20 μM physostigmine. ACh = acetylcholine.

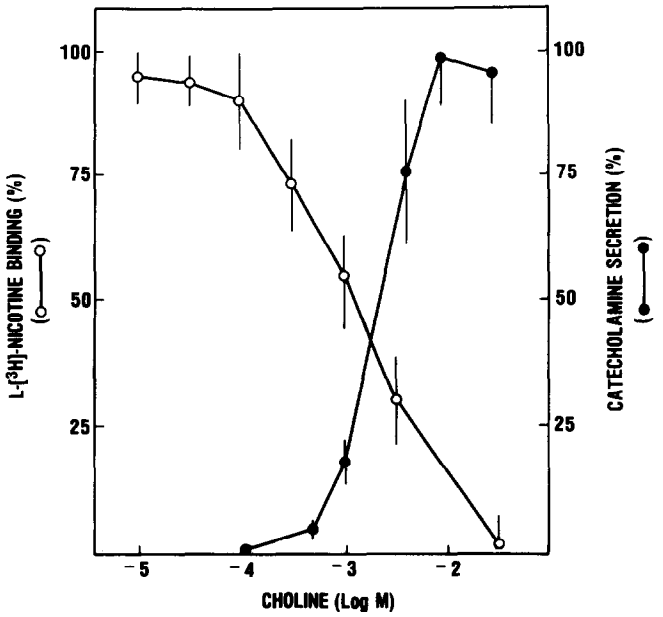

Fig. 2. Effects of choline on catecholamine secretion (●——●) from the adrenal gland and on L-[^{3}H]nicotine binding (○——○) in adrenal medulla homogenates. For release experiments, the left adrenal gland was isolated and vascularly perfused with Krebs–Ringer buffer containing the indicated concentration of choline (0–32 mM). The perfusate was collected at 2-min intervals, and catecholamine levels were analyzed by fluorometric assay. Catecholamine release is expressed as ng/2 min and is presented as the percentage of maximum release (675 ± 75 ng/2 min; mean ± SE). The basal release rate was 3.1 ± 0.3 ng/2 min. For binding experiments, membrane preparations of the adrenal medulla were incubated with L-[^{3}H]nicotine (3.2 nM) for 40 min at 37° with various concentrations of choline chloride (0–32 mM). L-[^{3}H]nicotine binding at each choline concentration was expressed as the percentage of specific binding in the absence of added choline.

pletely block, the response to choline. However, 1 μM atropine had no effect whatsoever on the response to 5 mM choline and, when added to the perfusion medium at a concentration of 100 μM, produced only a 30% reduction in the choline-stimulated secretion of adrenal catecholamines. These results indicate that the effect of choline on catecholamine secretion is mediated solely by nicotinic receptors.

Potency of choline to displace [^{3}H]*QNB binding from membrane preparations.* The potency of choline to displace [^{3}H]QNB from muscarinic receptor binding sites was assessed by incubating tissue homogenates with a fixed concentration of [^{3}H]QNB (0.9 nM) and various concentrations (0.1 to 100 mM) of choline. Choline displaced [^{3}H]QNB binding in all of the tissues examined in a concentration-dependent manner although its potency differed greatly from tissue to tissue (Table 2). An IC_{50} value was determined graphically by log-probit analysis; a K_i was derived from this and previously determined [^{3}H]QNB dissociation constants. K_i values of choline are shown in Table 2.

The nature of choline's inhibition of [^{3}H]QNB binding to brain was characterized further by constructing a series of saturation curves for [^{3}H]QNB in the presence of 0, 3.2, 10.0 or 32.0 mM choline. Increasing the concentration of choline caused a parallel shift to the right of the concentration–occupancy curves of [^{3}H]QNB (Fig. 3); analysis by Schild plot gave a pA_2 value of 2.66 ± 0.12 (mean ± SE) with a slope of −1.07, suggesting that choline competes with [^{3}H]QNB to bind to muscarinic receptors following the law of mass action. The K_A was 2.19 ± 0.62 mM.

Potency of choline to displace L-[^{3}H]*nicotine binding from membrane preparations.* L-[^{3}H]Nicotine binding was saturable in all of the tissues examined, and specific binding was 65–85% of total binding. Scatchard analysis showed that K_d values for L-[^{3}H]nicotine binding were similar among brain regions and peripheral tissues (Table 3), but that B_{max} values varied, with the thalamus and diaphragm having the highest concentrations of binding sites (data not shown). Choline displaced L-[^{3}H]nicotine binding in all tissues although its inhibitory potency varied over a 3-fold range. In brain, detectable inhibition (5–10%) was first observed at a choline concentration of 0.32 mM, and half-maximal and maximal inhibition occurred at choline concentrations of 1 and 10 mM respectively. The potency of choline to inhibit L-[^{3}H]nicotine binding in peripheral tissues was within the range of values determined in brain.

Effect of choline administration on serum choline levels. To determine the concentration of choline attainable in serum, rats were treated with choline chloride and killed at various time intervals thereafter. The oral administration of choline in doses of 0.6, 1.2 or 2.4 g/kg increased serum choline from basal levels of approximately 10 μM to a maximum concentration of almost 200 μM 15 min after

Table 2. Potency of choline to inhibit [^{3}H]QNB binding to muscarinic receptors in rat brain and peripheral tissues

	K_i (μM)		Relative potency	
			Choline-Atrium	Acetylcholine
Tissue	Choline	Acetylcholine	Choline-Tissue	Choline
Whole brain	2390 ± 620		1.2	
Cortex	3540 ± 640	67.6 ± 17.5	0.8	52
Corpus striatum	1373 ± 720	119.3 ± 19.0	2.2	12
Cerebellum	2479 ± 690	5.2 ± 0.2	1.2	477
Hippocampus	1392 ± 670	60.7 ± 11.0	2.1	23
Hypothalamus	1231 ± 620	8.1 ± 1.1	2.4	152
Thalamus	3030 ± 640		1.0	
Brainstem	1260 ± 470	2.7 ± 0.2	2.4	467
Spinal cord	1767 ± 540		1.7	
SCG	1256 ± 700		2.4	
ICG	1409 ± 680		2.1	
Adrenal medulla	1405 ± 480		2.1	
Right atrium	2973 ± 113		1.0	
Left atrium	2928 ± 125		1.0	
Ventricle	344 ± 49		8.6	
Fundus	597 ± 78		5.0	
Ileum	415 ± 83		7.2	
Colon	2039 ± 122		1.5	
Urinary bladder	1014 ± 160		2.9	
Parotid gland	281		10.6	
Submaxillary gland	1418		2.1	
Pancreas	2509		1.2	

K_i values were determined from the equation $K_i = IC_{50}/(1 + H/K_d)$ where H = concentration of [^{3}H]QNB (0.9 nM). K_d values for [^{3}H]QNB binding were determined for individual brain regions and peripheral tissues and varied between 0.290 and 0.908 nM. The IC_{50} values were determined graphically by log-probit analysis. The IC_{50} of acetylcholine was determined in the presence of 10 μM eserine. The data are expressed as the mean ± SE of four animals except for the parotid and submaxillary glands and pancreas (N = 2). Each sample was assayed in duplicate. SCG = superior cervical ganglion; ICG = inferior cervical ganglion.

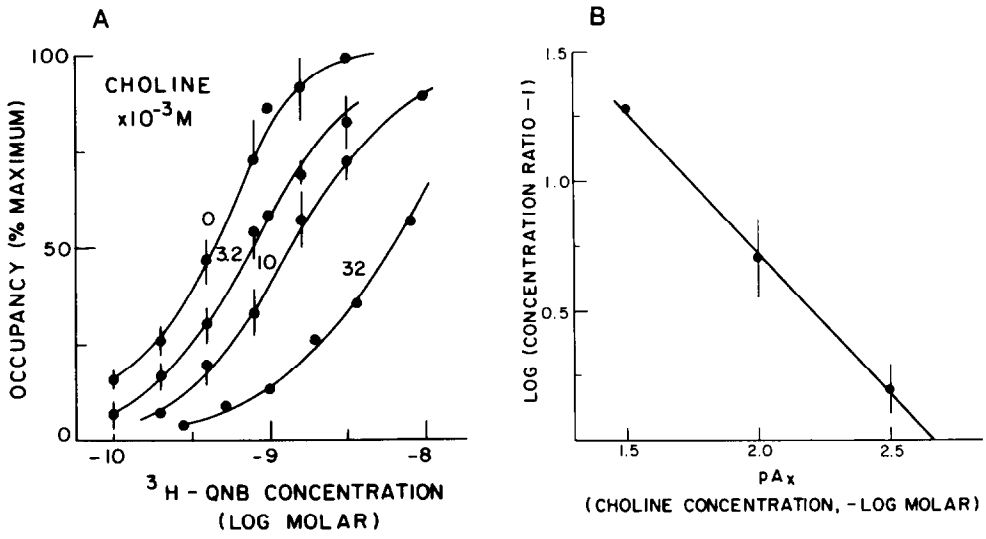

Fig. 3. Inhibition of [^{3}H]QNB binding to rat brain homogenates by choline. Panel A: Saturation analysis of [^{3}H]QNB binding to rat brain homogenates in the presence of 0, 3.2, 10.0 or 32.0 mM choline chloride. Panel B: Schild plot of the data presented in panel A.

Table 3. Inhibition of L-[^{3}H]nicotine binding by choline

Tissue	IC_{50} (μM)	K_i (μM)	K_d (nM)
Brain regions			
Cortex	589 ± 162	403 ± 111	6.9
Corpus striatum	708 ± 148	488 ± 102	7.1
Hypothalamus	550 ± 251	379 ± 173	7.1
Spinal cord	708 ± 151	521 ± 111	8.9
Thalamus	1540 ± 114	1167 ± 104	10.0
Hippocampus	1096 ± 128	726 ± 100	6.3
Brainstem	1479 ± 208	973 ± 137	6.1
Sympatho-adrenal system			
Adrenal medulla	1122 ± 176	805 ± 125	8.0
SCG	933 ± 286	666 ± 204	7.7
ICG	882 ± 337	624 ± 237	7.9
Skeletal muscle			
Diaphragm	835 ± 135	575 ± 93	7.1

Membrane preparations from rat tissues were incubated with L-[^{3}H]nicotine (3.2 nM) for 40 min at 37° with various concentrations of choline (0–32 mM). The concentrations which inhibited L-[^{3}H]nicotine binding by 50% (IC_{50}) were estimated graphically using a logit transformation. Inhibition constants (K_i) were calculated from the equation $K_i = IC_{50}/(1 + H/K_d)$ where H = the concentration of L-[^{3}H]nicotine (3.2 nM). The values shown are the mean ± SE of three separate experiments for brain regions or four to eleven experiments for peripheral tissues; each sample was assayed in duplicate. SCG = superior cervical ganglion; ICG = inferior cervical ganglion.

treatment with the highest choline dose (Table 4). Serum choline levels remained elevated for several hours and returned to control levels within 6–24 hr after treatment.

Intraperitoneal injection of 30, 60 or 120 mg/kg of choline chloride also produced a marked and dose-related increase in serum levels of choline. Serum choline levels returned to control values within 30 min after the intraperitoneal administration of 30 mg/kg choline but remained elevated 2- to 4-fold above baseline levels 60 min after the administration of 60 or 120 mg/kg of choline (Table 4). The maximum serum choline concentration observed after intraperitoneal choline administration was approximately 200 μM, similar to that produced by oral administration.

DISCUSSION

The present experiments examined the agonistic potency of choline on cholinergic receptors in rat brain and peripheral tissues to determine how much choline must be administered *in vivo* or applied to isolated tissue preparations *in vitro* in order to produce a distinct cholinergic response. The results of this study indicate that choline binds to both muscarinic and nicotinic receptors and produces biological responses in very high micromolar and low millimolar concentrations although its potency varied markedly among different brain regions and peripheral tissues.

Choline was most potent at producing a biological response in the fundus among the tissues investigated

Table 4. Effect of choline administration on serum choline concentrations: Dose- and time-course of the effect

Dose	Serum choline concentration (μM)					
Oral administration	15 Min	30 Min	1 Hr	3 Hr	6 Hr	24 Hr
Control	11.6 ± 2.0	10.8 ± 0.6	10.9 ± 2.3	8.3 ± 0.8	9.3 ± 0.7	9.8 ± 0.5
0.6 g/kg	20.9 ± 3.3	23.6 ± 3.5	22.9 ± 1.8	39.4 ± 10.8	27.0 ± 10.4	10.6 ± 1.6
1.2 g/kg	64.5 ± 9.2	34.1 ± 5.3	44.4 ± 11.9	52.8 ± 9.4	81.7 ± 16.9	10.7 ± 1.6
2.4 g/kg	185.0 ± 54.0	51.7 ± 5.2	68.3 ± 9.4	95.2 ± 8.5		10.5 ± 2.0
Intraperitoneal administration	5 Min	10 Min	15 Min	20 Min	30 Min	60 Min
Control	9.7 ± 0.7	10.1 ± 0.8	9.4 ± 1.4	10.1 ± 0.7	12.7 ± 3.0	9.7 ± 2.2
30 mg/kg	29.4 ± 2.7	43.8 ± 4.2	30.2 ± 3.0	25.3 ± 3.1	11.0 ± 2.1	8.9 ± 1.3
60 mg/kg	100.0 ± 27.9	102.0 ± 12.5	81.0 ± 15.3	37.5 ± 1.5	30.0 ± 7.5	20.6 ± 4.1
120 mg/kg	187.0 ± 61.5	192.7 ± 35.6	195.0 ± 54.0		52.0 ± 5.6	41.5 ± 9.1

Groups of three to seven rats were treated with choline chloride either orally (by stomach tube) or by intraperitoneal injection and killed at the indicated time interval. Serum choline concentrations were determined by radioenzymatic assay [14]. The data are expressed as the mean ± SE.

in the present study. It produced a measurable contraction of isolated strips of fundal smooth muscle at a concentration as low as 100 μM (Fig. 1). The potency of choline in the fundus was similar to its potency in the guinea pig ileum as previously reported ($pD_2 = 3.74 \pm 0.02$ [9] or 3.20 ± 0.07 [18]). Choline was an extremely weak agonist in the urinary bladder and right atrium of rats. When present at a sufficient concentration, however, choline produced the same maximal contraction of the urinary bladder as acetylcholine and completely inhibited the spontaneous beating of the right atrium indicating that choline, like acetylcholine, is a full agonist. The concentration of choline which produced either a significant contraction of isolated strips of urinary bladder smooth muscle or a decrease in the spontaneous beating of the atrium was about 1 mM (Fig. 1). The pD_2 value of choline in the atrium (1.84 ± 0.18) agrees with a previous report from this laboratory [9] and was similar to the pD_2 value for the urinary bladder (1.96 ± 0.15) (Table 1). The agonistic potency of choline in the isolated smooth muscle of rat trachea ($pD_2 = 2.75 \pm 0.15$) was substantially different, however; it was higher than the atrium and urinary bladder and lower than the rat fundus and guinea pig ileum. The potency of choline, relative to the atrium, was 1.3, 8.4 and 35.5 in the urinary bladder, trachea and fundus respectively (Table 1).

It has been shown previously that choline competitively displaces [^{3}H]QNB binding to membrane preparations of rat brain [10, 11] and cerebral cortex [12]; its IC_{50} is approximately 2 mM. The present results further extend these earlier findings by examining the ability of choline to compete for the binding of [^{3}H]QNB in specific brain regions. We found that K_i values of choline varied over a 3-fold range from 1.23 mM in the hypothalamus to 3.54 mM in the cortex. K_i values determined in the corpus striatum, hippocampus and brainstem were comparable to that of the hypothalamus but lower than those for the cortex, thalamus and cerebellum (Table 2). These results clearly demonstrate that the affinity of muscarinic receptors for choline in brain exhibits a distinct regional heterogeneity.

The potency of choline to inhibit [^{3}H]QNB binding to peripheral muscarinic receptors also varied considerably among different tissues (Table 2). Choline's potency was lowest in the atrium and highest in the fundus, in agreement with its potency to produce a biological response in these tissues. The K_i of choline for displacing [^{3}H]QNB from membrane preparations of the fundus (597 ± 78 μM) was not significantly different than the EC_{50} value of the drug (410 ± 125 μM) for eliciting contractions of this tissue. Thus, the concentration–receptor occupancy curve of choline was similar, if not identical, to the concentration–response curve of choline in this tissue. Choline displaced [^{3}H]QNB binding from homogenates of the parotid gland and ventricle with a potency similar to that observed in the fundus and ileum. In all other peripheral tissues, including the adrenal medullae, sympathetic ganglia, atria, submaxillary salivary gland, pancreas, colon and urinary bladder, the K_i values for choline were higher than 1 mM (Table 2). The potency of choline in the right and left atria, lowest among the peripheral tissues tested, was similar to its potency in several regions of brain, including the cerebral cortex, cerebellum and thalamus. The affinities of choline for muscarinic receptors in the adrenal medullae and sympathetic ganglia were comparable and were also within the range of K_i values determined in the corpus striatum, brainstem, hippocampus and hypothalamus.

When these results are viewed collectively, it is apparent that the potency of choline to bind to muscarinic receptors and/or produce a muscarinic type biological response in different tissues can be roughly divided into three different concentration ranges, although there is considerable overlap. In some tissues, such as the fundus, ileum, parotid gland and the ventricles of the heart, choline was effective in a concentration range of 0.1 to 1.0 mM. In other tissues, including the sympathetic ganglia, adrenal medullae, corpus striatum, brainstem and trachea, higher choline concentrations (0.5 to 10 mM) were required. For a third group of tissues, the affinity of choline for muscarinic receptors was still lower (1 to 100 mM); these included the atrium, pancreas and several regions of brain. The concentration of choline in serum was normally 10–20 μM, far below that required to activate muscarinic receptors in any of the tissues examined herein. Serum choline levels can be increased several-fold, up to 200 μM, by intraperitoneal injection [2] (Table 4), intravenous infusion [19] or by oral administration [7, 20] (Table 4) of exogenous choline. Exogenous choline administration produces considerably smaller changes in brain tissue, raising choline levels from approximately 30 μM to 60–70 μM [2, 19, 20]. The ingestion of a choline-enriched diet also elevates circulating choline levels but only to approximately 70 μM [21]. These concentrations are much lower than those necessary for choline to bind to a significant population of muscarinic receptors either in the central nervous system or in most peripheral tissues of the rat. In addition, because cholinergic nerve terminals possess a high affinity choline transport system [22], the concentration of choline at muscarinic receptors is likely to be far lower than serum levels. On the other hand, however, significant muscarinic effects may be expected at these concentrations in some parts of the gastrointestinal canal. Indeed, hypersalivation and diarrhea, which are usually observed following the administration of choline, particularly when given by gavage, may result from the direct stimulation of muscarinic receptors by choline.

Choline also activates presynaptic muscarinic receptors and, thereby, inhibits acetylcholine release from myenteric plexus-longitudinal muscle preparations of the guinea pig ileum [23]. The EC_{50} of choline for inhibiting acetylcholine release from the guinea pig ileum (300 μM) is similar to its EC_{50} for stimulating contractions of this tissue (180 μM) [9]. Thus, elevated circulating choline levels may not only activate postsynaptic muscarinic receptors in some peripheral tissues but also inhibit the release of acetylcholine by interacting with presynaptic receptors. In the central nervous system, pre- and postsynaptic muscarinic receptors are also equivalent in their affinities for agonists [24] and so exogenous

choline is unlikely to activate either pre- or postsynaptic receptors.

The present results also demonstrate that choline displaced L-[^{3}H]nicotine binding to membrane preparations of rat brain and peripheral tissues which indicates that choline, itself, binds to nicotinic cholinergic receptors albeit with a low affinity. The apparent affinity of choline for nicotinic receptors in brain (K_i = 379–1167 μM) (Table 3) was, as in the case of muscarinic receptors, considerably higher than the choline concentration of brain tissue either under control conditions or following the administration of exogenous choline [2, 19, 20]. Thus, it appears unlikely that brain choline concentrations can achieve levels necessary to activate nicotinic receptors in the central nervous system. On the other hand, serum choline levels approached the range of K_i values in peripheral tissues (575–805 μM) following the administration of exogenous choline (200 μM) (Table 4), suggesting that circulating choline may attain levels sufficient to directly activate nicotinic receptors in the adrenal medulla, sympathetic ganglia and neuromuscular junction.

Here we have shown that choline activates nicotinic receptors in the adrenal medulla, thus stimulating catecholamine release from the vascularly perfused adrenal gland. Choline's EC_{50} for releasing catecholamines (2.1 mM) was comparable to its EC_{50} for displacing L-[^{3}H]nicotine binding to membrane preparations of the adrenal (1.3 mM) (Fig. 2), further indicating that L-[^{3}H]nicotine binding assays provide a reasonable estimate of the affinity of choline for nicotinic receptors. However, the minimum choline concentration necessary to increase significantly the secretion of adrenal catecholamines (0.5 mM) was higher than the maximum choline concentration attainable in serum. Holz and Senter [25] have also shown that choline stimulates catecholamine secretion from primary cultures of bovine adrenal chromaffin cells, apparently by acting as a partial nicotinic agonist. Here again, the choline concentration necessary to stimulate secretion (1–10 mM) was considerably higher than circulating choline levels. These results question whether choline can directly stimulate catecholamine release *in vivo*. Previous studies from this laboratory have shown that choline does indeed activate adrenal nicotinic receptors *in vivo* but it does so by a transsynaptic mechanism and not as a direct receptor agonist [7, 9]. These experiments showed that choline administration to rats induces adrenal medullary tyrosine hydroxylase activity but, unlike classical nicotinic receptor agonists, such as nicotine, choline has no effect on tyrosine hydroxylase activity following splanchnic nerve transection. These results indicate that choline induces tyrosine hydroxylase activity indirectly, by increasing acetylcholine release, and not by directly activating nicotinic receptors.

Hutter reported, over 35 years ago, that choline activates nicotinic receptors at the neuromuscular junction, producing a prolonged depolarizing neuromuscular blockade when administered by close intra-arterial injection in high doses (20–50 mg/kg) [26]. Choline was ineffective, however, when given at a much lower dose (0.3 mg/kg) which elevated serum concentrations to the range attainable after oral administration (approximately 140 μM). This dose of choline increased the amplitude of end-plate potentials and overcame the neuromuscular blockade produced by *d*-turbocurarine, suggesting to the author that choline increases the output of acetylcholine from motor nerve terminals [26]. The direct measurement of acetylcholine release from rat hemidiaphragm preparations has also demonstrated that relatively low choline concentrations (30–60 μM) augment the amount of acetylcholine released from motor nerve terminals by phrenic nerve stimulation [27]. Together, these studies suggest that elevated levels of circulating choline may facilitate cholinergic neurotransmission at nicotinic receptors by a trans-synaptic mechanism but it does not act as a direct nicotinic agonist either because synaptic choline levels are too low or, conceivably, as suggested by earlier investigators, because choline acts, not as an agonist, but as an antagonist or mixed agonist/antagonist at nicotinic receptors [25, 28, 29].

It is well known that the acetylation of choline enormously enhances (200–100,000 times) its muscarinic and nicotinic activities [9, 11, 12, 15, 18, 30]. In the present study, we found that acetylcholine was 1,600, 47,000 and 66,000 times more potent than choline in the fundus, urinary bladder and atrium respectively (Table 1). Acetylcholine is approximately 100 and 1000 times more potent than choline in displacing [^{3}H]QNB binding in cortical [12] and whole brain [10, 11] homogenates respectively. We observed, however, that the potency of acetylcholine for competing with [^{3}H]QNB binding varied over a 30- to 50-fold range among brain regions; its potency, relative to choline, was 12, 23, 52 and 467 in the corpus striatum, hippocampus, cortex and brainstem respectively. The potency ratio of acetylcholine : choline reported here is similar to that determined by Krnjevic *et al.* [31] in electrophysiologic studies. They found that choline has an excitatory action when applied iontophoretically to hippocampal cells which also responds to the application of acetylcholine; both effects are blocked by atropine, indicating that they are mediated by muscarinic receptors. The potency ratio of acetylcholine : choline in the hippocampus was about 10–20 in their studies, in good agreement with our measured ratio of 23.

The most striking feature of the agonistic potency of choline was, perhaps, its wide range of threshold concentrations. To explain the observed variations in the muscarinic potency of choline and classical muscarinic agonists among different tissues of the rat, we initially suggested that at least two muscarinic receptor subtypes might be present [9, 32]. Evidence for the existence of muscarinic receptor subtypes, M_1 and M_2, has since been reported [33]. Moreover, it also appears that M_2 receptors exhibit heterogeneous binding properties [34]. Thus, variations observed in the muscarinic potency of choline may be attributable to the varying abundance of M_1 and M_2 receptor subtypes or to the varying affinity of M_2 receptors for choline.

In conclusion, the results of the present study clearly show that choline has a low affinity for both muscarinic and nicotinic receptors. Under *in vivo*

conditions, the affinity of choline for muscarinic and nicotinic receptors appears to be far too low for it to contribute significantly to the cholinergic effects of choline in the central nervous system. The direct activation of cholinergic receptors in peripheral tissues, however, may account for some of the side effects produced by choline administration.

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