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Muscarinic receptor localization and function in rabbit carotid body

B.G. Dinger, L. Almaraz, T. Hirano, K. Yoshizaki, C. Gonzalez, A. Gomez-Niño and S.J. Fidone

Department of Physiology, School of Medicine, University of Utah, Salt Lake City, UT 84108 (U.S.A.)

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Acetylcholine and muscarinic agonists inhibit chemosensory activity in the rabbit carotid sinus nerve (CSN). Because the mechanism of this inhibition is poorly understood, we have investigated the kinetics and distribution of muscarinic receptors in the rabbit carotid body with the specific muscarinic antagonist [^{3}H]quinuclidinylbenzilate ([^{3}H]QNB). Equilibrium binding experiments identified displaceable binding sites (1 μM atropine) with a K_d = 71.46 pM and a B_{max} = 9.23 pmol/g tissue. These binding parameters and the pharmacology of the displaceable [^{3}H]QNB binding sites are similar to specific muscarinic receptors identified in numerous other nervous, muscular and glandular tissues. Comparisons of specific binding in normal and chronic CSN-denervated carotid bodies suggest that muscarinic receptors are absent on afferent terminals in the carotid body; however, nearly 50% of the specific [^{3}H]QNB binding is lost following chronic sympathectomy, suggesting the presence of presynaptic muscarinic receptors on the sympathetic innervation supplying the carotid body vasculature. Autoradiographic studies have localized the remainder of [^{3}H]QNB binding sites to lobules of type I and type II parenchymal cells. In separate experiments, the muscarinic agonists, oxotremorine (100 μM) and bethanechol (100 μM) were shown to inhibit both the release of catecholamines and the increased CSN activity evoked by nicotine (50 μM) stimulation of the in vitro carotid body. Our data suggest that muscarinic inhibition in the rabbit carotid body is mediated by receptors located on type I cells which are able to modulate the excitatory actions of acetylcholine at nicotinic sites.

INTRODUCTION

The carotid body is an arterial chemosensory organ whose parenchyma consists of two distinct cell types in close association with a dense capillary network. Type I (glomus) cells possess abundant dense and clear cored synaptic vesicles, mitochondria and a highly conspicuous rough endoplasmic reticulum. Specialized terminals formed by afferent fibers from the carotid sinus nerve (CSN) synaptically appose the type I cells while slender cytoplasmic processes of type II cells, whose cytology resembles that of Schwann cells, envelop the afferent terminal-type I cell complex. Presumably, responses in the CSN produced by exposure of the carotid body to hypoxia, hypercapnia or low pH result from the release of a transmitter from type I cells which excites the adjacent afferent nerve terminals.

Early pharmacological studies by Eyzaguirre and his colleagues (see ref. 14 for review) of the actions of acetylcholine (ACh) in the mammalian carotid body were performed primarily in the cat, and revealed that nicotinic agonists excite the chemoreceptors and potentiate the response to the natural stimuli, hypoxia and hypercapnia (see ref. 14 for review). Later investigations with the cat carotid body demonstrated the presence of ACh[16,17], choline acetyltransferase[1,2,37] and acetylcholinesterase[25,26] in the specialized lobules of carotid body parenchymal (type I) cells. The afferent fibers innervating the organ, on the other hand, were shown to be nearly devoid of cholinergic activity[16,19]. Concurrent with many of these observations, the Loewi-type experiments of Eyzaguirre and his colleagues[11,12] led them to suggest that an 'ACh-like' substance was released as a sensory transmitter from the type I cells of the cat carotid body to act on the synaptically apposed afferent nerve terminals. However, since the formulation of this cholinergic hypothesis of chemotransmission, numerous studies (see ref. 14) have demonstrated the presence and release of multiple neuroactive substances from the type I cells of cat, rabbit and rat carotid bodies, including catecholamines (dopamine and norepinephrine) and neuropeptides (substance P and Met-enkephalin). The present-day concept that has thus evolved envisions some or all of these neuroactive agents acting in concert to determine the net sensory output of the organ. Amongst such possible interactions, evidence has accumulated that ACh

Correspondence: S.J. Fidone, Department of Physiology, University of Utah School of Medicine, 410 Chipeta Way, Research Park, Salt Lake City, UT 84108, U.S.A.

release might be coupled to the release of catecholamines[24]. Indeed, recent biochemical and autoradiographic studies have demonstrated that nicotinic (α-bungarotoxin) binding sites are present on the catecholamine-containing type I cells in the cat carotid body[6], and furthermore, that the nicotine- and hypoxia-evoked release of dopamine, and the accompanying increase in sensory discharge, are substantially reduced in the presence of 50 nM α-bungarotoxin[6].

A unified interpretation of these data has unfortunately been confounded by the more recent findings that ACh is inhibitory to chemoreceptor activity in the *rabbit* carotid body[8,29], in contrast to its well-known excitatory actions in the cat. Pharmacological studies demonstrated that the chemosensory inhibition in the rabbit is mediated by muscarinic receptors[8,29]. However, the location of these receptors in the carotid body has not been examined, and consequently the mechanism of muscarinic inhibition is unknown.

In the present study, we have used the muscarinic antagonist, [^{3}H]quinuclidinylbenzilate ([^{3}H]QNB), to quantify and localize muscarinic receptors in the rabbit carotid body. Biochemical and autoradiographic data indicate that specific binding of [^{3}H]QNB in rabbit carotid body, like that for α-bungarotoxin in the cat carotid body, is localized to lobules of type I cells and is absent from the afferent terminals of the carotid sinus nerve (CSN). Companion studies utilizing cholinergic agonists and antagonists demonstrate that muscarinic receptors located on type I cells are able to inhibit both CSN discharge and the release of catecholamines from these cells evoked by nicotine.

A preliminary report of some of the autoradiographic observations was published earlier in this journal[7].

MATERIALS AND METHODS

Adult New Zealand white rabbits of both sexes were used in these experiments. In addition to normal, unoperated (control) animals, two groups of rabbits were chronically denervated 12–15 days prior to the experiments. Under pentobarbital anesthesia (40 mg/kg, i.v.) and with aseptic surgery, the CSN were resected bilaterally in one group of 7 rabbits, while in a second group of 6, the superior cervical ganglia (SCG) were removed bilaterally along with their ganglioglomerular nerves to the carotid body.

Binding assay

Methodological details of the binding experiments have been published previously[7]. Briefly, carotid bodies were rapidly removed from normal or chronically denervated animals and dissected free of encapsulating connective tissue in ice-cold 100% O_2-equilibrated, modified Tyrode's solution (in mM: NaCl, 112; KCl, 4.7; $CaCl_2$, 2.2; $MgCl_2$, 1.1; sodium glutamate, 42; HEPES, 5; pH 7.43 at 37 °C with glucose 1 mg/ml). Wet weights were determined on a Cahn electrobalance equipped with a humidified chamber to prevent drying of the tissues. The tissue samples were transferred to glass scintillation vials containing 1 ml of modified Tyrode's solution, placed in a waterbath-shaker (100 Hz) and preincubated for 20 min at 37 °C in the presence or absence of competing drugs (10^{-10} to 10^{-3} M atropine, QNB, bethanechol or nicotine). A subsequent 60-min incubation period began with the addition of 100 μl of media containing [^{3}H]QNB (33.1 Ci/mmol; New England Nuclear). Following incubation, the tissue samples were washed in the shaker bath for 90 min in 2 ml of fresh buffer at room temperature. For determination of radioactivity, tissue samples were combusted in a Packard Model 306 sample oxidizer, solubilized in 15 ml of Oxifluor and counted in a Parkard Model 3385 scintillation spectrometer.

Autoradiography

The carotid bifurcation with the carotid body was quickly removed from the animal, and the vasculature flushed with 3 ml of ice-cold oxygenated buffer via the common carotid artery. Preincubation and incubation times were reduced to 10 and 20 min, respectively, to enhance histological preservation, and the normal wash media was replaced with phosphate buffered 1% paraformaldehyde and 1% glutaraldehyde. Tissue samples were post-fixed for 1 h in 1% OsO_4, dehydrated in a graded series of ethanols and embedded in Araldite. Semithin sections (0.5–1.0 μm) were mounted on gelatin-subbed glass slides, dipped in a 1:1 aqueous dilution of Kodak NTB-2 emulsion and stored with desiccant at 4 °C for 8 months. Autoradiographs were developed in Dektol (2.5 min at 18 °C), stained with methylene blue and photographed through a Zeiss Universal Microscope. Autoradiographic grain density over parenchymal cell lobules was determined in 2000× color photographs in which the episcopically illuminated silver grains appeared as unambiguous amber dots overlying the methylene blue-stained tissue. The photographs were number coded and evaluated in a 'double-blind' paradigm. The areas of parenchymal cell lobules were subsequently estimated by trimming and weighing the photographs, each of which included one or two cell lobules.

Release studies

The effects of muscarinic agents on the release of [^{3}H]catecholamines and CNS discharge were determined according to previously published methods[15]. Briefly, pairs of carotid bodies were incubated for 2 h in 25 μM [^{3}H]tyrosine (spec. act. 40–50 Ci/mmol), after which the organs were mounted in a tiny platinum wire basket suspended within a closed, temperature- and humidity-controlled chamber. The CSN from one carotid body was drawn up into an adjacent suction-type electrode. With the preparation in place, the carotid bodies were directly beneath an array of 22-gauge hypodermic needles which were connected through a peristaltic pump to reservoirs containing unlabeled modified Tyrode's solution. The solutions were preheated and gas-equilibrated in the reservoirs; final heating of the solutions to 37 °C was accomplished with a servocontrolled heating system. The temperature of the drop surrounding the carotid body was directly monitored through a second thermistor which was positioned to abut against the basket. The relative humidity in the chamber was maintained at 100% by a constant flow of gas, saturated with water vapor. Under control and stimulus conditions, the superfusates were equilibrated with 100% O_2. The stimulus solutions contained nicotine (50 μM) either alone or in the presence of a muscarinic agonist (bethanechol or oxotremorine, 10^{-4} M), which was introduced 10 min before stimulation. The volumes of superfusate per collection period varied less than 5%. The superfusion rate in all experiments was 0.4 ml/min and was established in preliminary experiments which assessed the viability of the preparation, judged on the basis of a stable chemoreceptor response over a 5 h superfusion period.

The action potentials from the CSN nerve were led through an AC-coupled preamplifier to an oscilloscope and a magnetic tape recorder. The amplified signals were also led through a window discriminator to: (1) a frequency-to-voltage converter for final display of the spontaneous neural activity on a chart recorder; and (2) a digital counter-printer which read the total number of nerve impulses recorded during the collection period.

The superfusates were collected in vials containing a carrier so-

lution consisting of 0.3 M acetic acid, 1 mM ascorbic acid, at a final pH of 3.6. The solutions were processed with the alumina adsorption method, and [^{3}H]catecholamines ([^{3}H]CA) were batch eluted from columns with 1 N perchloric acid[38]. Radioactivity was determined in a Packard Model 1500 liquid scintillation counter.

In separate experiments, [^{3}H]CA release was evaluated in carotid bodies incubated in superfusion media contained in glass vials, as described previously[21].

RESULTS

Time course of [^{3}H]QNB association and washout from the tissue

Preliminary experiments established conditions for washout of unbound [^{3}H]QNB from intact carotid bodies. After 45 min of incubation in 400 pM [^{3}H]QNB, carotid bodies were washed for up to 2 h with continuous agitation in 2 ml of buffer at room temperature. Specific binding (displaceable by atropine, 10^{-6} M) remained stable during this rinse period, as might be expected from the reported high affinity of [^{3}H]QNB for muscarinic receptor sites[4,41,42]. In subsequent experiments, carotid bodies were washed for 90 min because retention of non-specific binding was not reduced with longer rinse periods.

Separate experiments established the association time course for 150 pM [^{3}H]QNB, where non-specific binding in the presence of 10^{-6} M atropine equilibrated rapidly and remained at a stable value, accounting for less than 10% of total binding for up to 60 min. Specific binding (total binding minus non-specific binding) rose sharply during the initial 30 min and then plateaued. Subsequent equilibrium studies utilized 45 min incubation periods in order to achieve steady-state conditions with lower concentrations (<150 pM) of [^{3}H]QNB.

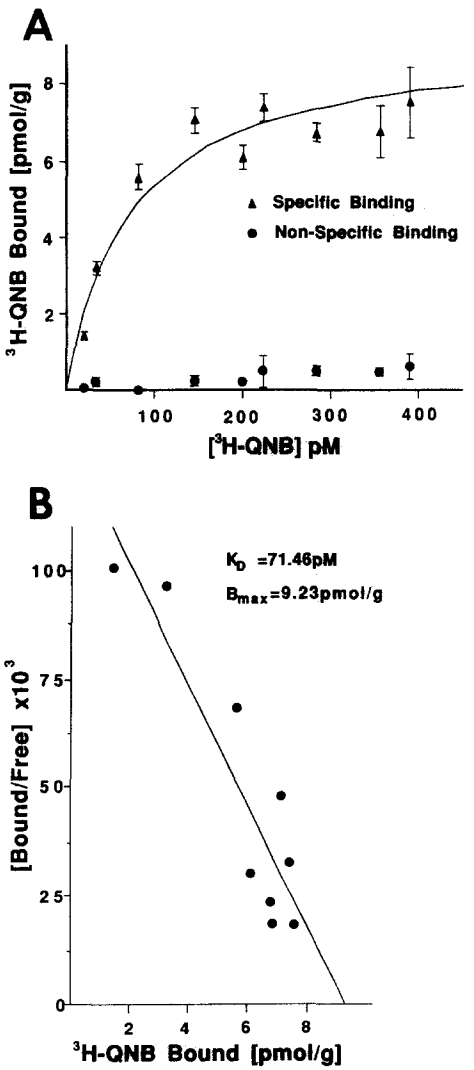

Fig. 1.A: equilibrium binding data from rabbit carotid bodies incubated for 45 min in [^{3}H]QNB at various concentrations. Each point represents $\bar{X}\pm$ S.E.M. for 3–7 catotid bodies. Total binding not shown. B: Scatchard analysis for a single population of binding sites estimates a K_d = 71.46 pM and a B_{max} = 9.23 pmol/g of tissue.

Equilibrium kinetics and pharmacology of [^{3}H]QNB binding

Specific [^{3}H]QNB binding in rabbit carotid body is characterized by high affinity and saturability. Fig. 1A shows specific and non-specific binding at [^{3}H]QNB concentrations between 20 and 390 pM. Specific binding rose sharply at low concentrations of [^{3}H]QNB and plateaued at concentrations above 200 pM. Non-specific binding in the presence of 10^{-6} M atropine never exceeded 10% of total binding, and increased linearly over the concentration range of [^{3}H]QNB. In Fig. 1B, a Scatchard analysis of the binding data suggests the existence of a homogeneous population of binding sites with a K_d of 71.46 pM and a B_{max} of 9.23 pmol/g of carotid body tissue. These K_d values are similar to those for [^{3}H]QNB binding to muscarinic receptors in mammalian brain (K_d = 60 pM[41]), sympathetic ganglion (K_d = 830 pM[4]) and ileum (K_d = 30 pM[42]).

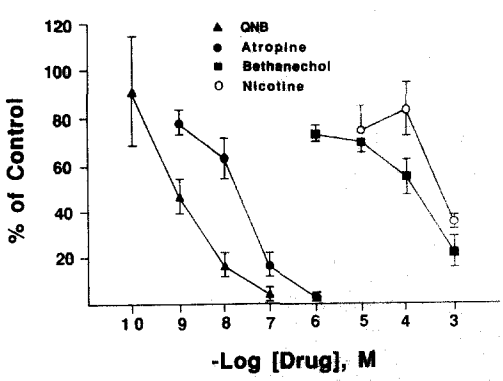

Fig. 2. Inhibition of [^{3}H]QNB binding. Competing drugs were introduced into the superfusion media 20 min prior to incubation in [^{3}H]QNB (150 pM). Incubation continued with each competing drug for 45 min followed by a 90 min wash in fresh media. Each point represents data ($\bar{X}$ ± S.E.M.) from 4–6 carotid bodies.

Competitive inhibition studies of [^{3}H]QNB binding further confirmed the muscarinic nature of the binding site. Fig. 2 illustrates the relative potency of 4 competing drugs. The muscarinic antagonists, QNB and atropine, were potent inhibitors of [^{3}H]QNB binding in the carotid body, while the agonist, bethanechol, significantly reduced binding only at concentrations above 10^{-5} M. Nicotine also significantly decreased binding at very high concentrations (10^{-3} M), but was ineffective at 10^{-4} and 10^{-5}. The order of potency of these competing drugs for the [^{3}H]QNB binding site is similar to that demonstrated in other structures where [^{3}H]QNB binds with high affinity to specific muscarinic receptors[4,23,41,42].

Localization of specific [^{3}H]QNB binding sites

As mentioned earlier, the putative chemosensory tissue of the mammalian carotid body consists of lobules of type I (glomus) and type II (sustentacular) cells, together with the associated sensory nerve terminals. In addition, the organ also receives a significant sympathetic innervation from the nearby SCG. In order to distinguish muscarinic binding sites on parenchymal cells versus afferent or autonomic nerve terminals, we examined [^{3}H]QNB binding in carotid bodies following chronic section of the CSN or removal of the SCG.

Fig. 3 shows that 12–15 days following resection of the CSN, the number of [^{3}H]QNB binding sites is unchanged from normal unoperated tissue. In contrast, after removal of the SCG and degeneration of the sympathetic terminals in the carotid body, [^{3}H]QNB binding is reduced by 46%. These data suggest that muscarinic receptors are absent from CSN afferent fibers and terminals, but that sympathetic axons contain nearly one-half of carotid body muscarinic sites. An important question is whether the remaining [^{3}H]QNB binding following sympathectomy is associated with the lobules of type I and type II parenchymal cells. The autoradiographic localization of muscarinic binding sites was examined in carotid bodies after incubation in 400 pM [^{3}H]QNB. Fig. 4A,B presents typical autoradiographs showing silver grain distributions over parenchymal cells in two normal carotid body lobules. In these preparations, silver grains are clearly associated with the lobules of type I and type II cells. In the presence of 10^{-6} M atropine (Fig. 4C, normal), grains are sparse over the tissue.

Quantification of the grain density overlying lobules of type I and type II cells from normal, CSN-denervated and sympathectomized tissue samples confirmed the data from our binding experiments (Fig. 5). Under all 3 conditions, tissues incubated with 10^{-6} M atropine (non-specific binding) displayed uniformly low grain counts. Similarly low 'background' counts were measured over the lumen of blood vessels ($3.81 \times 10^{-3} \pm 0.89 \times 10^{-3}$ grains/μm^2; not shown in the figure). Non-specific [^{3}H]QNB binding in the autoradiographs was therefore minimal, which agrees with our binding data. In the absence of atropine (i.e. total binding), grain counts were significantly higher in all preparations ($P < 0.005$), reflecting specific [^{3}H]QNB binding. These quantitative autoradiographic data suggest that specific binding within parenchymal cell lobules was unchanged following either chronic CSN denervation or chronic removal of the SCG (i.e. total binding was not significantly different for the 3 conditions, $P > 0.10$). Thus, degeneration of the afferent or sympathetic innervation to the carotid body does not alter the density of [^{3}H]QNB binding sites within the parenchymal cell lobules.

The effects of muscarinic agents on catecholamine release and CSN discharge

The effects of oxotremorine on catecholamine release and chemoreceptor discharge of the CSN were tested using carotid bodies preloaded with [^{3}H]CA synthesized from [^{3}H]tyrosine (see Materials and Methods). The organs with their attached nerves were mounted in the in vitro recording chamber, and superfused with 100% O_2-equilibrated media for 30–40 min prior to stimulation. [^{3}H]CA release and CSN discharge were then monitored during sequential 5 min prestimulus, stimulus and poststimulus collection periods, which together comprised a single 'stimulus cycle'. Following a 'control' nicotine-

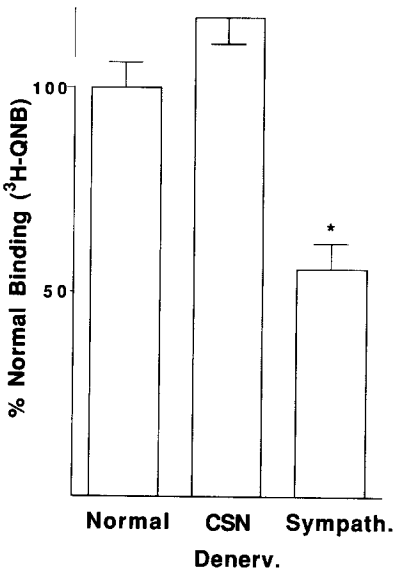

Fig. 3. Specific [^{3}H]QNB binding ($\bar{X} \pm$ S.E.M.) in normal carotid bodies and following chronic (12–15 days) resection of the carotid sinus nerve (CSN Denerv.) or removal of the superior cervical ganglion (Sympath.). Binding for each group was determined under equilibrium conditions (45 min incubation) in the presence of 200 pM [^{3}H]QNB. Each bar represents data from at least 4 pairs of carotid bodies incubated in the presence or absence of 1 μM atropine. *$P < 0.0005$ vs normal group.

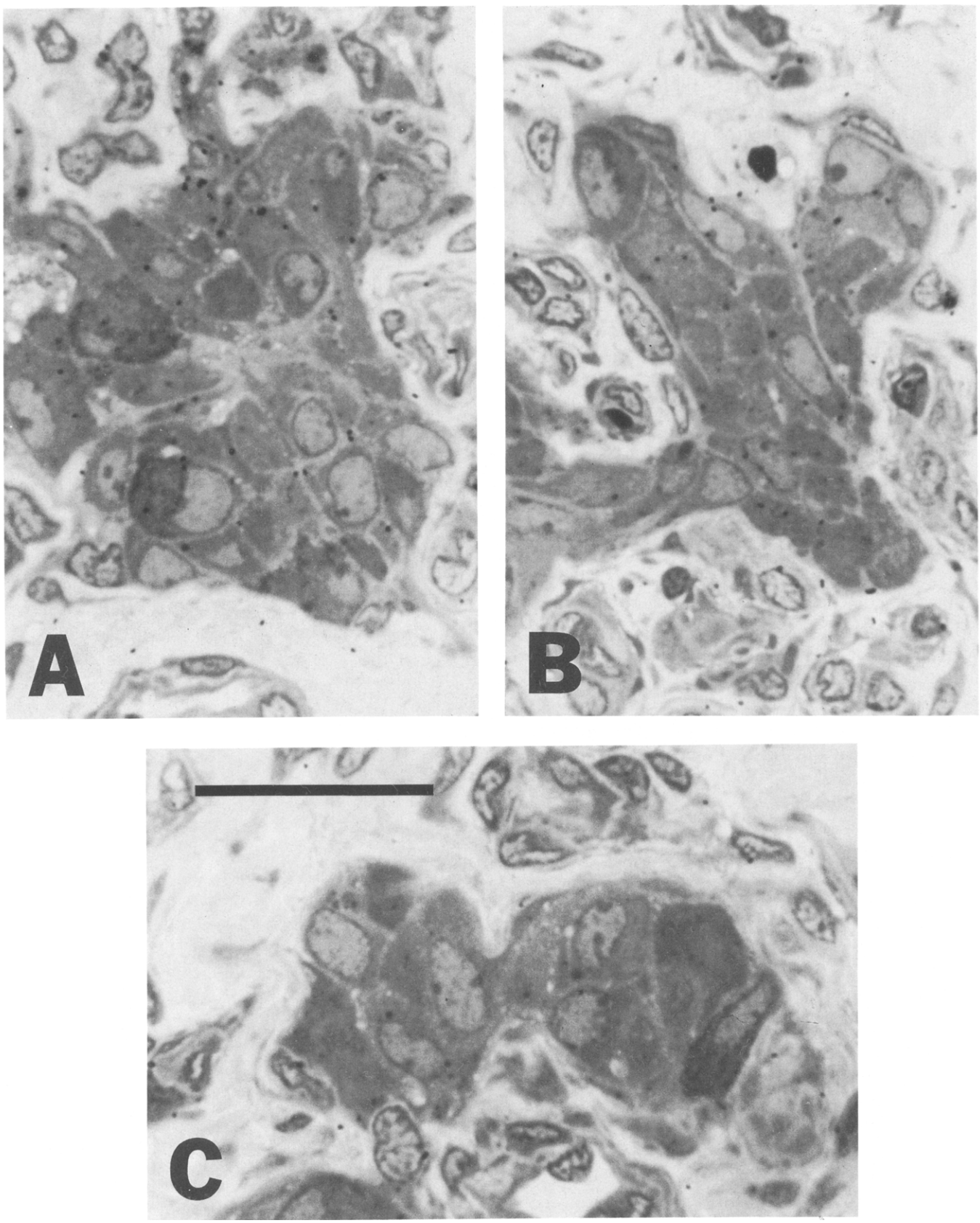

Fig. 4. Autoradiographic distribution of [^{3}H]QNB binding in lobules of type I and type II cells in rabbit carotid bodies; (A and B) normal, (C) normal carotid body incubated in the presence of 1 μM atropine. Autoradiographic grains appear as black punctate dots. Scale bar = 20 μm.

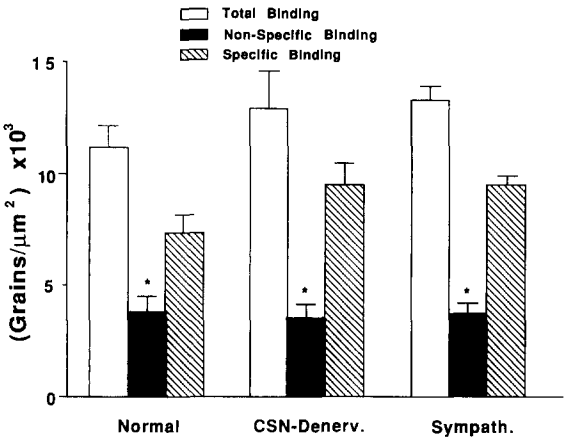

Fig. 5. Quantitative autoradiographic data ($\bar{X}$ ± S.E.M.) of [^{3}H]QNB binding in lobules of type I and type II cells in normal, CSN-denervated (12–15 days) and sympathectomized (12–15 days) carotid bodies. Silver grain density was determined in carotid bodies incubated in the presence (non-specific binding) or absence (total binding) of 1 μM atropine. *$P < 0.0005$ vs total binding. See text for methodological details.

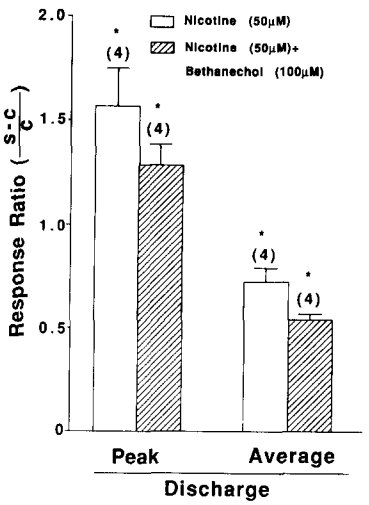

Fig. 7. Effect of bethanechol (10^{-4} M) on CSN activity evoked by nicotine (50 μM). *$P < 0.05$ compared to response evoked by nicotine alone. Other details as in Fig. 6.

stimulus cycle, the preparation was superfused for 5 min with 100% O_2 media containing 10^{-4} M oxotremorine, and then re-exposed to nicotine (50 μM) in the presence of the muscarinic drug. The data in Fig. 6 show [^{3}H]CA release and CSN discharge expressed as response ratios, i.e. (stimulus minus control)/control, where a value of 1 would indicate a 100% increase in release (see ref. 15). Nicotine, in the absence of muscarinic drugs, increased [^{3}H]CA release by 125%. Recordings of CSN activity likewise showed that nicotine evoked large increases in both peak and average CSN discharge during the 5 min stimulus period. In contrast to these nicotinic effects, oxotremorine did not alter basal [^{3}H]CA release and CSN discharge. However, in the presence of oxotremorine, [^{3}H]CA release and CSN discharge evoked by nicotine (50 μM) were significantly reduced; the response to nicotine returned to near control values after a 1 h wash with Tyrode's solution (100% O_2-equilibrated). In other experiments which evaluated drug effects only on nerve activity (Fig. 7), the muscarinic agonist bethanechol was shown to inhibit the nicotine-evoked increase in CSN discharge ($P < 0.05$).

The effects of bethanechol on the nicotine-evoked re-

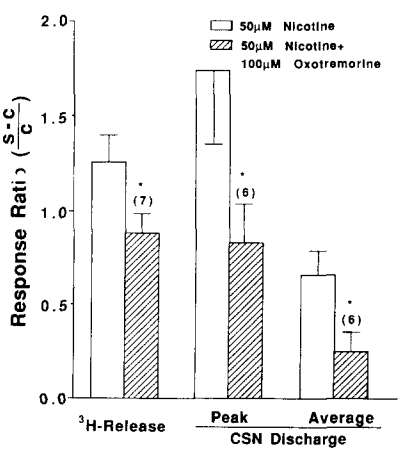

Fig. 6. Effects of oxotremorine (100 μM) on the nicotine (50 μM) evoked release of [^{3}H]CA and CSN discharge in the rabbit carotid body. The data are expressed as response ratios [$(S–C)/C$], where S represents the [^{3}H]CA released, or the nerve activity, measured during the stimulus period; C is the control value determined during an equivalent time period immediately prior to the stimulus. See ref. 15 for further explanation. CSN discharge was evaluated both for its peak response, as well as the averaged activity over the 5-min stimulus period. Values in parentheses equal number of measurements. *$P < 0.01$ compared to the data obtained with nicotine alone.

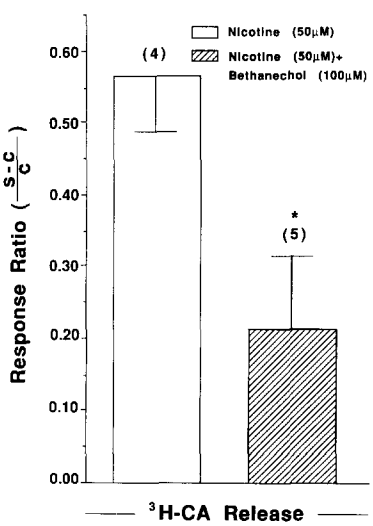

Fig. 8. Effect of bethanechol (10^{-4} M) on [^{3}H]CA release evoked by nicotine (50 μM) in rabbit carotid bodies superfused in glass vials. Values in parentheses equal number of observations. *$P < 0.025$ compared to [^{3}H]CA release evoked by nicotine alone. Response ratio explained in legend to Fig. 6.

lease of [^{3}H]CA was examined in a slightly different experimental protocol, in which carotid bodies were superfused in glass vials. The organs were stimulated with nicotine (50 μM) in the presence or absence of this muscarinic agonist. Fig. 8 shows that bethanechol (10^{-4} M) inhibited the nicotine-evoked release of [^{3}H]CA (expressed as a response ratio) by more than 60%, thus paralleling our results for the nicotine-evoked CSN activity obtained with the superfusion-recording chamber. Collectively, these data clearly demonstrate that muscarinic agonists can negatively modulate nicotinic actions in the rabbit carotid body.

DISCUSSION

In the present study, we have attempted to localize muscarinic receptors in the rabbit carotid body, and to examine their functional relationship to the physiological response of this arterial chemoreceptor organ. Our principal findings suggest that muscarinic receptors are located on the specialized type I parenchymal cells, and that they modulate the nicotine-evoked release of catecholamines from these cells.

The equilibrium binding data indicate that [^{3}H]QNB associates with a single population of binding sites in the carotid body with a K_d of 71.46 pM, which is similar to the high affinity binding of [^{3}H]QNB to muscarinic receptors in brain[41], sympathetic ganglion[4], ileum[42], heart[23], coronary artery[39], and adrenal medulla[31]. Moreover, the order of potency of competing drugs for the [^{3}H]QNB binding sites is consistent with the pharmacology of muscarinic receptors in these other tissues, where specific antagonists (QNB and atropine) possess a high affinity for the receptor, in contrast to the low affinity displayed by agonists such as bethanechol[23,40–42]. Although we have not performed additional binding experiments using pirenzepine to define the muscarinic receptor subtype[3,9,22,27], we have found in preliminary experiments that bethanechol reduces the increase of cAMP produced by forskolin in the carotid body, suggesting a negative coupling of muscarinic receptors to adenylate cyclase; in other structures negative coupling occurs via M_2 or M_3 muscarinic receptors[20,30].

Because chemosensory transduction is thought to occur within the lobules of the type I and type II parenchymal cells (see ref. 14), our experiments were designed to elucidate the location of putative muscarinic receptors associated with these lobules. Sensory fibers from the CSN contribute an important functional element to the chemoreceptor apparatus, namely the specialized terminals which form synaptic appositions with the type I cells[18,33]. In our experiments, CSN-denervation did not reduce the amount of specific [^{3}H]QNB binding in rabbit carotid bodies, and a quantitative autoradiographic analysis of binding within the parenchymal cell lobules likewise failed to reveal changes in binding after CSN degeneration. These findings imply that muscarinic receptors are absent from the sensory fibers and their terminals. However, because these nerve terminals represent less than 5% of the total volume of the carotid body[32], a small reduction in [^{3}H]QNB binding associated with CSN-denervation might not have been detected in our experiments. While this remains a possibility, it is perhaps not consistent with the observed 46% reduction in [^{3}H]QNB binding following degeneration of sympathetic fibers to the carotid body, which likely also represent a similarly small volume of tissue.

The 46% reduction of [^{3}H]QNB binding in rabbit carotid body after removal of the SCG can be attributed to the loss of presynaptic muscarinic receptors associated with the axon terminals of postganglionic sympathetic neurons. In other sympathetically innervated tissues, the presence of presynaptic muscarinic receptors was shown in pharmacological experiments which demonstrated the ability of muscarinic agents to inhibit [^{3}H]norepinephrine release[39]. While our biochemical experiments show a profound reduction in total organ binding following sympathectomy, our autoradiographic data from sympathectomized carotid bodies suggest that binding is unchanged within lobules of type I and type II cells. This observation is consistent with morphological studies which demonstrate that sympathetic axons rarely terminate within the parenchymal cell lobules of the carotid body[36], but instead form abundant nerve endings on the smooth muscle of the carotid body vasculature[10,28,36].

The function of muscarinic receptors in the carotid body can be inferred from our data showing that muscarinic agents inhibit the release of [^{3}H]CA and CSN discharge evoked by nicotine. Earlier studies by Monti-Bloch and Eyzaguirre[29] and by Docherty and McQueen[8] showed that muscarinic drugs (ACh and pilocarpine) inhibit chemosensory units of the CSN. Likewise, our neurochemical and electrophysiological data clearly demonstrate that muscarinic receptors are negatively coupled to nicotinic excitation of the rabbit carotid body.

The mechanism of interaction of nicotinic and muscarinic receptors in the carotid body is unknown. In many other species, both nicotinic and muscarinic agents independently evoke the release of CA from the adrenal medulla. However, in bovine adrenal chromaffin cells, it has been reported that muscarinic agents inhibit CA release evoked by nicotine[5,35]. In this species, muscarinic agents activate guanylate cyclase, thus increasing the levels of cyclic GMP in medullary cells[43]. Furthermore, cGMP analogs and phosphodiesterase inhibitors mimick the muscarinic effect by decreasing CA release in the

presence of nicotine[5]. These findings suggest that muscarinic actions which are negatively coupled to nicotinic effects may be mediated by classical second messenger systems and may involve protein phosphorylation. Indeed, it has been established that phosphorylation of nicotinic receptors by a variety of protein kinases accelerates receptor desensitization (see ref. 34). Preliminary experiments in our laboratory have in fact shown that sodium nitroprusside (1.0 mM), an activator of soluble guanylate cyclase, depresses the CA release evoked from the rabbit carotid body by nicotine (50 μM).

The physiological role of ACh and nicotinic/muscarinic receptors in the mechanisms of chemoreception remain ill understood, however. Early experiments by Eyzaguirre and his collaborators demonstrated that an ACh-like substance is released from the carotid body during natural stimulation, most probably from the type I cells of the chemosensory lobules[11,12]. The findings in the present study suggest that ACh released by these cells may act at nicotinic autoreceptors to enhance CA release, while muscarinic autoreceptor activation modulates these effects. Consistent with this schema are preliminary observations in our laboratory which show that bethanechol (10^{-4} M) can inhibit the increase in [^{3}H]CA release and CSN discharge evoked by hypoxic stimulation[13].

In summary, a study of [^{3}H]QNB binding in rabbit carotid body has revealed that the type I cells possess specific muscarinic receptors. Muscarinic receptors are also associated with the sympathetic innervation to the organ, while in contrast, they appear to be absent from the chemosensory CSN terminals. While the precise role of muscarinic receptors in the chemoreceptor response to natural stimuli is not fully understood, studies of their effects on nicotine evoked CA released and CSN discharge suggest that muscarinic receptors are able to modulate the actions of ACh at nicotinic sites in the rabbit carotid body.

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