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Heterogeneity of neuronal nicotinic acetylcholine receptors in 5-HT-containing chemoreceptor cells of the chicken aorta

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1 The effects of nicotinic agonists and antagonists on whole-cell currents and 5-hydroxytryptamine (5-HT) release were studied in order to characterize nicotinic ACh receptors on the 5-HT-containing chemoreceptor cells of the chicken aorta.

2 ACh, nicotine and dimethylphenylpiperazinium (DMPP) evoked concentration-dependent inward currents accompanied by increases in current noise at a holding potential of −70 mV. The peak amplitude of the current response to DMPP was 50% larger than that to either nicotine or ACh.

3 Hexamethonium, α–bungarotoxin (α–BTX) and methyllycaconitine decreased nicotine-induced inward currents in a concentration-dependent manner. Although hexamethonium (0.1 mM) abolished the current response to nicotine (30 μM), a high concentration (1 μM) of α–BTX decreased it only by about 30% of the control response. Methyllycaconitine (0.1 μM) decreased the current response to nicotine to the same extent as did α–BTX whilst a high concentration (10 μM) abolished the response.

4 ACh, nicotine and DMPP caused concentration-dependent increases in 5-HT output from the thoracic aorta which effect was blocked by hexamethonium (0.1 mM). Pre-treatment with α–BTX (1 μM) for 30 min reduced the output of 5-HT induced by ACh to 70% of the control response.

5 It is suggested that neuronal nicotinic ACh receptors, sensitive and insensitive to α–BTX, are present on the chemoreceptor cells of the chicken aorta, the activation of which causes the release of 5-HT.

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Abbreviations: ACh, acetylcholine; α–BTX, α–bungarotoxin; DMPP, dimethylphenylpiperazinium; C_6, hexamethonium; 5-HT, 5-hydroxytryptamine

Introduction

It is well known that the carotid body senses arterial PO_2, PCO_2 and pH. Type I cells in the carotid body are thought to play an important role in sensing these changes and thereafter in releasing catecholamines, which are then capable of activating sensory nerve endings of the carotid sinus nerves (González *et al*., 1994). In addition to the carotid body, there are other peripheral chemoreceptor organs such as the aortic body located in the aorta. The electrophysiological characteristics of the chemoreceptor cells of the aorta are not well understood.

However, quite recently, Neutral Red-positive epithelioid cells containing 5-HT isolated from chicken thoracic aorta were subjected to whole-cell patch clamp, and it was found that the cells containing 5-HT exhibit voltage-dependent Na^+, K^+ and N-and L-type Ca^{2+} channels as well as oxygen-sensitive K^+ channels (Ito *et al*., 1999). As these characteristics of epithelioid cells in the thoracic aorta are similar to those of type I cells of the carotid body (Duchen *et al*., 1988; López-Barneo *et al*., 1988), it is suggested that these 5-HT-containing epithelioid cells are chemoreceptor cells (Ito *et al*., 1997; 1999). However, it has also been reported that carotid and aortic chemoreceptors differ not only in their sensitivity to certain stimuli such as normochronic anaemia, hypoxia and acidosis (Sampson & Hainsworth, 1972; Hatcher *et al*., 1978; Lahiri *et al*., 1981; Pokorski & Lahiri, 1983) but also in the distribution patterns of amine-containing cells in the tissues (Kummer & Addicks, 1986).

ACh is proposed to be an excitatory neurotransmitter released from type I cells in the rat carotid body (Zhong *et al*., 1997). Furthermore, ACh released from type I cells causes depolarization presynaptically of the type I cells (Nurse & Zhang, 1999). Nicotinic agonists have been shown to evoke inward currents (Wyatt & Peers, 1993) and Ca^{2+} transients (Dasso *et al*., 1997) in isolated type I cells of the carotid body. However, it remains unclear whether aortic chemoreceptor cells respond to nicotinic stimulation.

It has been reported that different α and β subunits contribute to the pharmacological and functional diversity of neuronal nicotinic ACh receptors (McGehee & Role, 1995) and that α–bungarotoxin (α–BTX) binding to α7 and α8 subunits of the ACh receptors inhibits receptor activation (Clarke, 1992). Methyllycaconitine has also been shown to be a selective α7 subunit antagonist at lower concentrations (Ward *et al*., 1990; Alkondon *et al*., 1996) but not at higher concentrations (Drasdo *et al*., 1992). The effects of nicotinic

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agonists on epithelioid cells in the aorta are potentially of interest since two types of neuronal nicotinic ACh receptors, sensitive and insensitive to α–BTX, are evident in carotid body chemoreceptor cells of the cat (Obeso *et al*., 1997).

The aim of the present experiments was to study the effects of cholinergic agonists such as ACh, nicotine and dimethylphenylpiperazinium (DMPP) on membrane currents in epithelioid cells isolated from the chicken thoracic aorta using the whole-cell patch clamp technique and on 5-HT release from the chicken thoracic aorta. In addition, the effects of α–BTX, hexamethonium or methyllycaconitine on membrane currents and 5-HT release in response to cholinergic agonists were also examined.

Methods

Tissue preparation

All experiments were carried out under the regulations of the Animal Research Committee of the Graduate School of Veterinary Medicine, Hokkaido University. Male chicks (White Leghorn, 14–18 days after hatching) were anaesthetized with O_2-bubbled ether and then decapitated. The thoracic aorta was removed and freed from surrounding tissues. The aorta was cut longitudinally to open its lumen and an aortic strip containing epithelioid cells was prepared by cutting it to about 4 mm in length. The pieces of the aortic tissue were kept in oxygenated physiological solution at 4°C until used.

Secretory experiments

A piece of aortic tissue was placed in physiological solution (0.1 ml) containing ACh, nicotine or dimethylphenylpiperazinium (DMPP) on ice, and then incubated at 37°C for 5 min. The solution used was of the following composition (mM): NaCl, 140; KCl, 4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; glucose, 10; HEPES, 10; pH 7.3 with NaOH. When the effects of hexamethonium (0.1 mM) and α–BTX (1 μM) on 5-HT release induced by agonists were examined, aortic tissues were pre-incubated for 5 min with hexamethonium and for 30 min with α–BTX at 37°C, respectively, and then incubated with the agonist in the continued presence of the corresponding blocker for another 5 min. The secretory response was terminated by placing the tube containing the tissue on ice. To measure the amount of 5-HT appearing in the incubation medium, the solution was treated with perchloric acid to a final concentration of 0.4 N after removal of the aortic tissue. The aortic tissue was also treated with 0.4 N perchloric acid to measure the amount of residual 5-HT. After centrifugation, supernatants were treated with K_2HPO_4, giving a final concentration of 290 mM (pH 4–6). After removal of potassium percolate, clear supernatant was applied to an h.p.l.c. system (an ODS-column; Catecholpak, Jasco Corp., Japan) equipped with an electrochemical detector (Eicom, Japan). The mobile phase was composed of the following: KH_2PO_4-H_3PO_4 buffer, 100 mM (pH 3.5); EDTA, 40 μM; sodium octasulfonic acid, 1.16 mM and methanol, 12–17% v v^{-1}. The release of 5-HT (per cent of content) was estimated by dividing the released 5-HT in the incubation medium by 5-HT content in the aortic tissue.

Epithelioid cell isolation

5-HT-containing epithelioid cells were isolated from three thoracic aortae with collagenase as described previously (Ito *et al*., 1999). In brief, the lumen of the thoracic aortae obtained from chicks (17–28 days after hatching) was treated with Ca^{2+}-free solution containing 0.15% w v^{-1} collagenase, 0.15% w v^{-1} soybean tyrosine inhibitor and 0.2% w v^{-1} bovine serum albumin for 30 min at 37°C. The enzymatic digestion was repeated twice using fresh enzyme solution. The cells isolated with the second enzyme digestion were resuspended in physiological solution containing 0.5 mM $CaCl_2$. The aliquot of the cell suspension was placed on coverslips and stored on ice under a 100% O_2 atmosphere until used. The coverslips were not coated with adhesive materials.

Membrane current measurement

The coverslip was placed in a chamber set on the stage of an inverted microscope (Diaphot 300, Nikon, Japan). The cells were perfused with physiological solution at a flow rate of 2–3 ml min^{-1}. In order to identify chemoreceptor cells, a vital dye, Neutral Red (20 or 30 μM), was applied for less than 1 min before starting the experiment. Neutral Red was used only to identify the cells and then was washed out. The resulting red-coloured cells were subjected to whole-cell voltage clamp. An antagonist (α–BTX, hexamethonium or methyllycaconitine) was applied to the cells *via* the bathing solution. Agonists (ACh, nicotine or DMPP) were applied to the cells using a Y-shaped tube that was connected to reservoirs of solutions containing the drugs and to a sink through a magnetic valve. The end of the Y-tube, having a hole of 250 μm in diameter, was placed about 1–2 mm from the cell of interest. The solution in the reservoir and chamber set on the microscope thus flowed to the sink by gravity as long as the valve was open. When the valve was shut, flow through the hole inverted and changed the solution around the cells.

Membrane current recording was performed by a standard patch clamp technique (Hamill *et al*., 1981) using an Axopatch 200A amplifier (Axon Ins., CA, U.S.A.). Whole-cell membrane currents were recorded with a heat-polished patch pipette (3.5–5 MΩ resistance) filled with an internal solution of the following composition (mM); CsCl, 140; $MgCl_2$, 1.2; Na_2 ATP, 2; EGTA-Na, 5; HEPES, 10; pH 7.2 with CsOH. The access resistance and membrane capacitance were 7.5–19 MΩ and 1.8–6.2 pF, respectively, both of which were estimated with the dial setting of the amplifier. The series resistance was not compensated. The voltage steps were produced by a step command generated by a microcomputer in conjunction with a Digidata 1200 interface using the program, pCLAMP version 6. (Axon). Data sampled at 100 Hz were stored on the hard disk of the computer using this interface. Data were also stored on the cassette tape of a PCM data recorder (NF Electronic Ins., Japan) and displayed on a pen-writing recorder (Recti-Horiz-8K, Sanei, Japan). Data are presented without correction for liquid junction potentials. Experiments were performed at room temperature, about 25°C, within 4 h after cell isolation. Graphic representation was performed using Microcal soft-

ware's Origin Technical Graphic Program (Northampton, U.S.A.). Curve fitting was carried out using this program. A single sigmoidal curve described by the following equation provided a good fit to the data:

$$F(x) = \frac{A_1 - A_2}{1 + (x/x_0)^n} + A_2$$

where x_0 is the EC_{50} for agonist, x being the concentration and n being the power, A_1 being the initial value when no agonist was used and A_2 being the maximal response value. A_1, a fitted value, did not become zero in some data. Therefore, the responses to the drugs at lower concentrations (1 and 10 nM) were fixed to 0 in order that the actual fitted value of A_1 turned out to be low.

Chemicals

Neutral Red, ACh, nicotine, dimethylphenylpiperazinium (DMPP) hexamethonium and α–BTX were purchased from Wako Pure Chem. Ltd. (Osaka, Japan) and methyllycaconitine from Tocris Cookson Inc. (Ballwin, U.S.A.). Collagenase (type I) and soybean tyrosine inhibitor (type 1) were obtained from Sigma-Aldrich Japan Co. (Tokyo, Japan) and bovine serum albumin (fraction V) was from Boehringer-Mannheim Co. (Tokyo, Japan).

Statistical analysis

All data are expressed as mean ± s.e.mean. Levels of statistical significance were calculated by analysis of variance (ANOVA) followed by Scheffé's test for multiple comparisons. The level of significance was set at $P<0.05$.

Results

Inward current responses to nicotinic agonists

Whole-cell current responses to ACh, nicotine and DMPP were examined in Neutral Red-positive cells at a holding potential of −70 mV. These drugs (5 s application) elicited inward currents. The repetitive application of the drugs caused reproducible inward currents at 3–5 min intervals, but run-down of the peak amplitude of the currents occurred. Therefore, the current response to nicotine (0.1 mM) was observed throughout the experiments as a control to normalize the response with other cholinergic agonists. The peak amplitude of nicotinic currents was decreased to 93.3 ± 1.9% ($n=9$) of the first response at 8–10 min, 84.9 ± 4.4% ($n=7$) at 16–18 min and 77 ± 4.8% ($n=5$) at 24–26 min. ACh and nicotine evoked concentration-dependent increases in inward currents in the same cell (Figure 1). The currents evoked by both drugs were always accompanied by increases in current noise. These drugs elicited inward currents without sag at lower concentrations. Increasing the concentrations produced a biphasic current response, that is, a transient inward current followed by sustained current decreasing gradually. Similar characteristics of the inward currents were observed with DMPP (0.3–100 μM) (traces not shown). The larger the transient peaks of inward currents, the

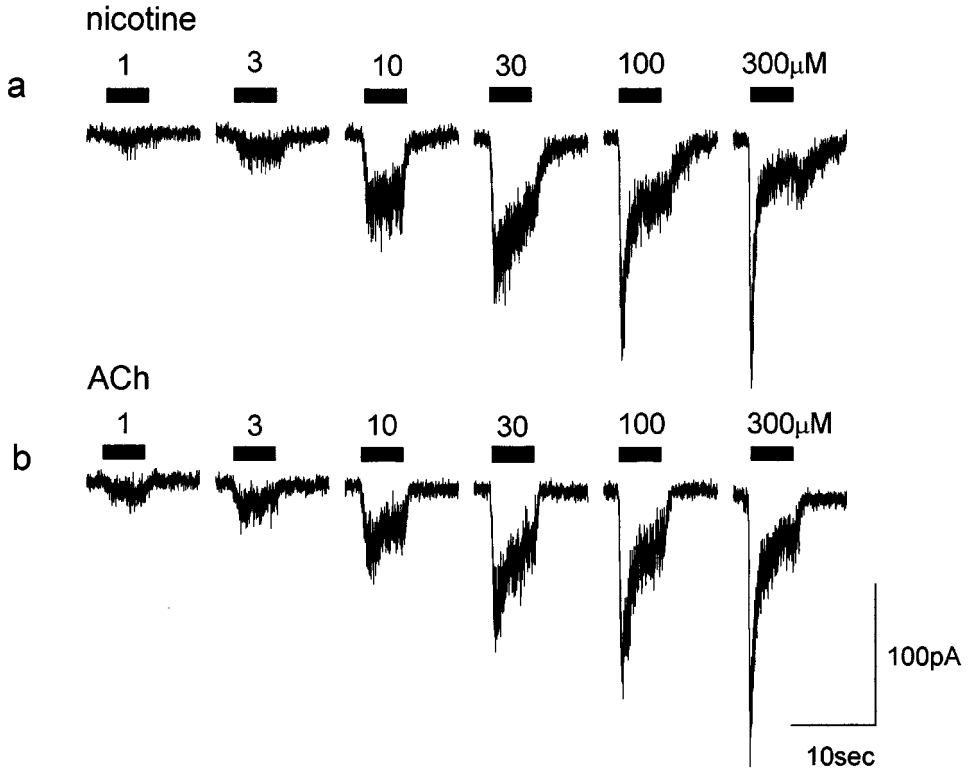

Figure 1 Inward currents evoked by nicotine and acetylcholine in a chicken aortic chemoreceptor cell. All traces were obtained from the same cell at a holding potential of −70 mV. The period of drug application is shown by horizontal bars. The concentrations used are shown above the horizontal bars.

greater the current sag, suggesting that high concentrations of ACh, nicotine and DMPP cause desensitization of the receptors.

The concentration-current response curves for ACh, nicotine and DMPP are shown in Figure 2. The peak amplitude of the inward currents induced by ACh or DMPP was expressed as a percentage of the mean amplitude of the peak currents induced by 0.1 mM nicotine obtained before and after the construction of concentration-response curve for each agonist. The response to 0.1 mM nicotine used in the concentration-response curve for nicotine was expressed as 100%. A full concentration-response curve was obtained from each cell. The peak amplitude of the inward current was increased with increasing concentrations of each drug. The peak amplitude of inward currents induced by the drug at 0.1 mM was -77.6 ± 16.8 pA ($n=7$) for ACh, -74.0 ± 7.7 pA ($n=18$) for nicotine and -93.0 ± 12.8 pA ($n=5$) for DMPP. The concentration-response curve for nicotine almost overlapped that for ACh. The peak amplitude of the current response to DMPP was 1.5 folds larger than that to nicotine or ACh. EC_{50} values were obtained from a concentration-response curve in each cell, separately. The mean EC_{50} values were 26.2 ± 4.9 μM ($n=7$) for ACh, 21.5 ± 8.7 μM ($n=6$) for nicotine and 8.5 ± 2.2 μM ($n=5$) for DMPP.

Pharmacological properties of inward current response to nicotine

The effects of hexamethonium, α-BTX and methyllycaconitine on the inward current response to nicotine were also examined. Nicotine (30 μM) was applied at 3–5 min intervals in the presence and absence of each blocker at various concentrations. These blockers were applied for 3–4 min before nicotinic stimulation. The nicotine-induced inward current was decreased by hexamethonium or methyllycaconitine in a concentration-dependent manner (Figure 3a,b). The effects of α-BTX and hexamethonium on the current response to nicotine at 30 μM in the same cell are shown in Figure 3c. α-BTX at 1 μM inhibited the current response to nicotine by 30% of the control response. More prolonged application of a lower concentration of α-BTX (15 min at 0.1 μM) had a similar effect, reducing responses to nicotine (30 μM) to 70.0 ± 4.2% of the control ($n=3$). The recovery from the inhibition by α-BTX was almost complete (91.3 ± 5.2%, $n=3$) in three cells 3–4 min after the washout of the toxin if the rundown was taken into account, but not in other cells (76.3 ± 5.0%, $n=3$), When nicotine was applied again to the three cells, 6–7 min after its washout, the current response almost recovered (87.3 ± 5.5%, $n=3$). When α-BTX were applied for 15 min, the response to nicotine partly recovered after 10 min in one cell, but did not in the other.

The inhibitory effects of hexamethonium, α-BTX and methyllycaconitine at various concentrations on the peak amplitude of the current response to nicotine (30 μM) are summarized in Figure 4. The peak amplitude of the current response in the presence of each blocker at each concentration was expressed as percentage of the mean amplitude of nicotine-induced currents before the blocker application and 5–8 min after its removal. Hexamethonium, α-BTX and methyllycaconitine inhibited the nicotine-induced inward current in a concentration-dependent manner. The inward currents were completely abolished by 0.1 mM hexamethonium and 10 μM methyllycaconitine but not 1 μM α-BTX. The concentration-inhibition curve for hexamethonium was parallel to that for methyllycaconitine at concentrations ranging from 0.1–10 μM, and methyllycaconitine at this concentration range was about 6 folds more effective than hexamethonium. The IC_{50} values for hexamethonium and methyllycaconitine were 1.7 and 0.3 μM, respectively.

Although hexamethonium and methyllycaconitine almost abolished the current response to nicotine, a high concentration (1 μM) of α-BTT only partly reduced the nicotine-induced inward current to 69.3 ± 2.2% of control response ($n=6$). The inhibition curve for α-BTX at concentrations ranging from 0.01–0.1 μM overlapped that for methyllycaconitine over the same concentration range.

Secretory responses to nicotinic agonists

The thoracic aorta was incubated with ACh, nicotine or DMPP at various concentrations for 5 min. These agonists caused increases in 5-HT output in the incubation medium in a concentration-dependent manner (Figure 5a–c). The effect of nicotine at 0.1 mM on 5-HT output was somewhat less than that of ACh and DMPP at the same concentration. Hexamethonium at 0.1 mM inhibited 5-HT output induced by 0.1 mM ACh from 8.9 ± 1.1% ($n=8$) to 2.9 ± 0.5% of content ($n=5$), 30 μM nicotine from 6.3 ± 0.9% ($n=11$) to 2.6 ± 0.4% ($n=5$) and 0.1 mM DMPP from 10.1 ± 1.0% ($n=8$) to 2.9 ± 0.6% ($n=5$). The effect of α-BTX on the secretory response to 0.1 mM ACh was examined after 1 μM α-BTX was pre-incubated for 30 min at 37°C. α-BTX significantly inhibited 5-HT release in response to ACh from the thoracic aorta only by about 30% ($P<0.05$).

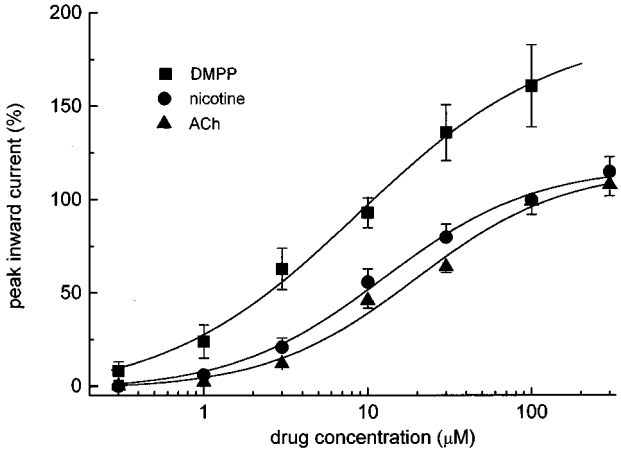

Figure 2 Log concentration-response curves for peak inward currents induced by nicotine, acetylcholine and dimethylphenylpiperazinium. Cells were voltage-clamped at -70 mV. The peak amplitude of the inward currents induced by ACh and DMPP was expressed as percentage of the mean amplitude of the peak currents induced by 0.1 mM nicotine obtained before and after the construction of concentration-response curve for each agonist except that the response to 0.1 mM nicotine used in the concentration-response curve for nicotine was expressed as 100%. Means and s.e.mean for nicotine ($n=6$) and ACh ($n=7$) and for DMPP ($n=5$) are plotted against a log concentration. A single sigmoidal curve described in the method provided a good fit to the pooled data. From the curve fitting, the EC_{50}, n and the maximal peak response are 19.3 μM, 0.96 and 116 for ACh, 12.3 μM, 0.94 and 118 for nicotine, 8.7 μM, 0.73 and 191 for DMPP, respectively.

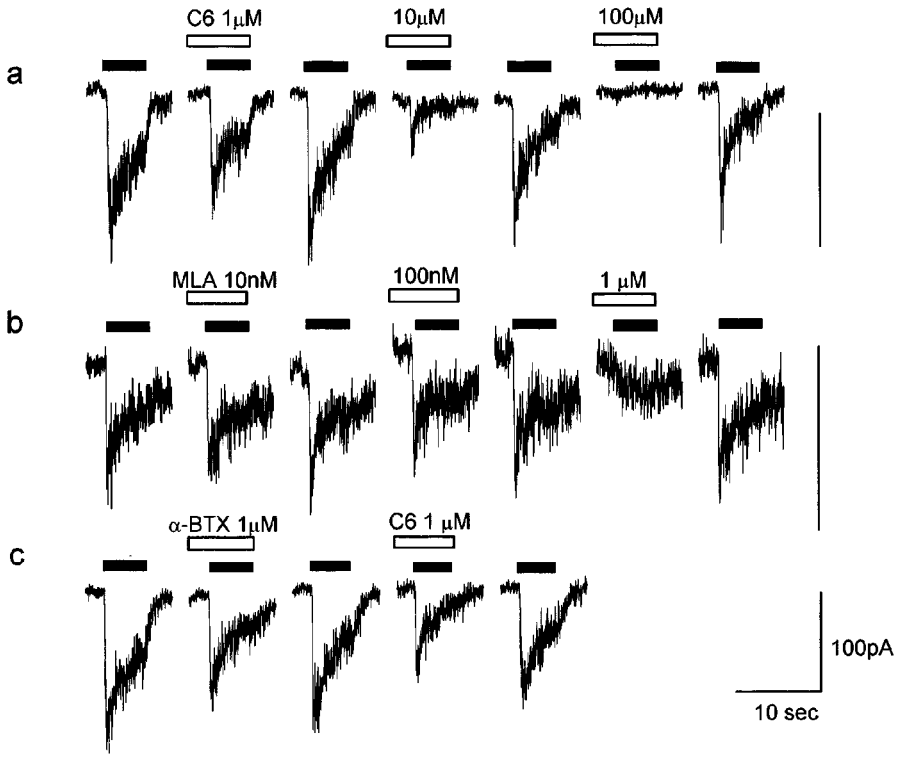

Figure 3 Inhibitory effects of hexamethonium, methyllycaconitine and α–bungarotoxin on nicotine-induced inward currents. The period of application of nicotine (30 μM) is shown by horizontal closed bars. Open horizontal bars indicate the application of blockers (3–4 min) at the concentration indicated above each bar. In (a), hexamethonium (C_6). In (b), methyllycaconitine (MLA). In (c), α–bungarotoxin (α–BTX) and C_6. In (a), (b) and (c), each result was taken from the same cell.

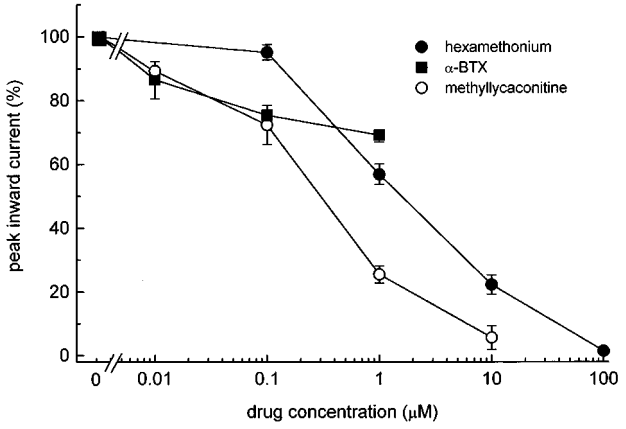

Figure 4 Concentration-dependent inhibition of the nicotine-induced inward currents by hexamethonium, methyllycaconitine and α–bungarotoxin. Data are expressed as a percentage of the mean peak value of nicotine (30 μM)-induced inward currents before blocker application for 3–4 min and after its washout. Means and s.e.mean for hexamethonium ($n=4-7$), methyllycaconitine ($n=4-6$, $n=3$ for 10 μM) and α–bungarotoxin (α–BTX, $n=5-6$, $n=3$ for 0.1 μM) are plotted against the log concentration of the blocker.

Discussion

The present results clearly indicate that neuronal nicotinic ACh receptors are present on the chemoreceptor cells of the chicken aorta because the activation of the receptors by ACh, nicotine and DMPP elicited inward currents and the release of 5-HT. The inward currents evoked by these drugs were accompanied by current noise and desensitization. In particular, these phenomena were prominent at high concentrations as reported for other preparations, including glomus type I cells of the carotid body (Wyatt & Peers, 1993). These results suggest that activation of neuronal nicotinic ACh receptors elicits depolarization of chemoreceptor cells of the aorta, resulting in the release of 5-HT. If this is the case, the chemoreceptor cells of the chicken aorta have characteristics similar to chemoreceptor type I cells of the mammalian carotid body.

Exposure to nanomolar concentrations of α–BTX for 30 min has been found to block rapidly desensitizing currents induced by nicotine through homomeric α7 channels subunits expressed in oocytes (Couturier *et al*., 1990). α–BTX irreversibly blocks rapidly desensitizing currents induced by ACh in rat hippocampal neurons (Alkondon & Albuquerque, 1993). Moreover, it has been proposed that three populations of native α7-containing neuronal nicotinic ACh receptors are distinct heteromeric complexes including other α and/or β subunits in embryonic chick sympathetic neurons (Yu & Role, 1998). In the cat carotid body, it has been reported that 30 min treatment with 100 nM α–BTX reduces nicotine-induced release of ^{3}H-catecholamine by 56%, suggesting the presence of two types of neuronal nicotinic ACh receptors which are sensitive and insensitive to α–BTX respectively (Obeso *et al*., 1997). In the chemoreceptor cells of the chicken aorta, treatment with 1 μM α–BTX for 3–4 min decreased the inward current response to nicotine by 30%, and the

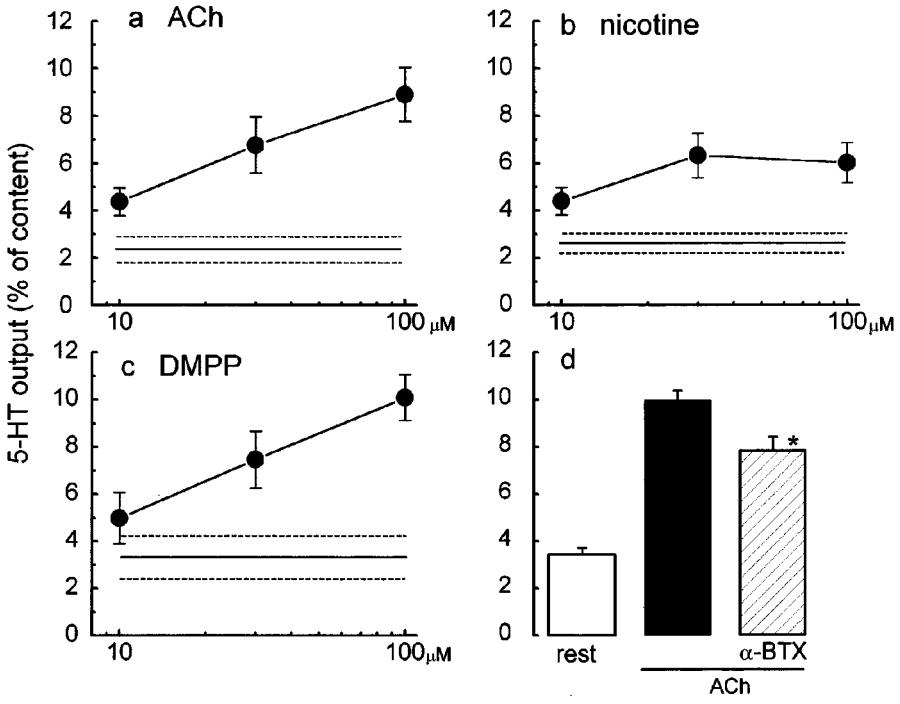

Figure 5 Concentration-response curves for ACh-, nicotine-, and dimethylphenylpiperazinium (DMPP)-induced 5-HT output and the effects of α–bungarotoxin (α–BTX) on ACh-induced 5-HT output from the thoracic aorta. The output of 5-HT is expressed as a percentage of the contents of 5-HT in the thoracic aorta. In (a), (b) and (c), the line and dotted lines in the concentration-response curve indicate the mean and s.e.mean of the resting levels ($n=4-5$), respectively. Symbols represents means ± s.e.mean for ACh (a, $n=5$), nicotine (b, $n=8-11$), and DMPP (c, $n=8$, $n=4$ for 10 μM). In (d), Open and solid columns indicate the means + s.e.mean of 5-HT output under the resting condition ($n=7$) and ACh-induced 5-HT output ($n=9$), respectively. Hatched columns represent mean + s.e.mean of ACh-induced 5-HT output from the tissue pretreated with α–BTX for 30 min ($n=15$), $*P<0.05$, significant difference from ACh-induced 5-HT output in the absence of α–BTX.

extent of this inhibition was almost the same as that which followed 15-min treatment. Furthermore, 5-HT release induced by ACh was only partly inhibited by 1 μM α–BTX. (30% inhibition of control). These results suggest that chicken aortic chemoreceptor cells possess two types of neuronal nicotinic ACh receptors, sensitive and insensitive to α–BTT. Similarly, at least two types of neuronal nicotinic ACh receptors are thought to be present on chick ciliary ganglion cells (Vijayaraghavan *et al*., 1992).

It has been shown that neuronal nicotinic ACh receptors expressed on oocytes exhibit functional differences among the subunit combinations (McGehee & Role, 1995). Quite recently, it has also been reported that some native neuronal nicotinic ACh receptors containing α7 subunit can behave atypically in rat superior cervical ganglion neurons when compared with homomeric α7 subunit expressed on oocyte and that the blockade of the nicotinic current by α–BTX occurs rapidly with a recovery which is almost complete within 5 min (Cuevas *et al*., 2000). In the present experiments, α–BTX rapidly inhibited current responses to nicotine, and the current inhibition by α–BTX recovered within 3–7 min after removal of the toxin. It seems likely that 'atypical' heteromeric receptors, containing other subunits in addition to α7, are present in the chemoreceptor cells of the chicken aorta. Further study is necessary to evaluate the molecular basis of the subunit composition.

It has been shown that nanomolar concentrations of methyllycaconitine inhibit ^{125}I-α–BTX binding to the α7 subunits of neuronal nicotinic ACh receptors and that micromolar concentrations inhibit ^{125}I-α–BTX binding to muscle nicotinic ACh receptors (Ward *et al*., 1990). In the present experiments, both α–BTX and methyllycaconitine at a concentration of 100 nM inhibited the current response to nicotine to the same extent (about 30%). These results suggest that methyllycaconitine blocks the same population of neuronal nicotinic ACh receptors composing α7 subunits as does α–BTX. However, micromolar concentrations of methyllycaconitine produced a further inhibition of nicotine-induced currents and the inhibition by methyllycaconitine was 6 folds more effective than that by hexamethonium. High concentrations of methyllycaconitine seem therefore to inhibit neuronal nicotinic ACh receptors other than those composing α7 subunits as reported by Drasdo *et al*. (1992).

It has been suggested that ACh may act as a transmitter released from type I cells in the carotid body (Dasso *et al*., 1997; Zhong *et al*., 1997; Nurse & Zhang, 1999). All aortic chemoreceptor cells examined responded to nicotine, indicating the presence of neuronal nicotinic ACh receptors on aortic chemoreceptor cells. However, the source of ACh that would activate these receptors *in vivo* is unclear. Further study is necessary to evaluate whether these cells release ACh like type I cells of the carotid body or they were innervated by cholinergic neurons.

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