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INVITED REVIEW

Human nicotinic receptors in chromaffin cells: characterization and pharmacology

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Abstract During the last 10 years, we have been working on human chromaffin cells obtained from the adrenal gland of organ donors that suffered encephalic or cardiac death. We first electrophysiologically characterized the nicotinic acetylcholine receptors (nAChRs) activated by acetylcholine, and their contribution to the exocytosis of chromaffin vesicles and release of catecholamines. We have shown that these cells possess an adrenergic phenotype. This phenotype may contribute to an increased expression of α7 nAChRs in these cells, allowing for recording of α7 nAChR currents, something that had previously not been achieved in non-human species. The use of α-conotoxins allowed us to characterize non-α7 nAChR subtypes and, together with molecular biology experiments, conclude that the predominant nAChR subtype in human chromaffin cells is α3β4* (asterisk indicates the posible presence of additional subunits). In addition, there is a minor population of αxβ2 nAChRs. Both α7 and non-α7 nAChR subtypes contribute to the exocytotic process. Exocytosis mediated by nAChRs could be as large in magnitude as that elicited by calcium entry through voltage-dependent calcium channels. Finally, we have also investigated the effect of nAChR-targeted tobacco cessation drugs on catecholamine release in chromaffin cells. We have concluded that at therapeutic concentrations, varenicline alone does not increase the frequency of action potentials evoked by ACh. However, varenicline in the presence of nicotine does increase this frequency, and thus, in the presence of both drugs, the probability of increased catecholamine release in human chromaffin cells is high.

Keywords Human · Chromaffin cells · Nicotinic receptors · α-conotoxins · Varenicline · Nicotine · Patch-clamp

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Introduction

Chromaffin cells of the adrenal gland are modified postganglionic sympathetic neurons, innervated by the splanchnic nerve, that control the release of catecholamines to the bloodstream. In humans, chromaffin cells have an adrenergic phenotype [34] and they are the primary source for adrenaline production and release. In the human adrenal gland, there is a juxtaposition of medulla and cortex (Fig. 1). This differs from other species such as cow, rat, or mouse, in which the separation between medulla and cortex is preserved. The cortex secretes high concentrations of glucocorticoids to the medulla, which activate the expression of the enzyme phenylethanolamine *N*-methyltransferase (PNMT) [51], and also the α7 subunit gene [9]. Transcriptional activators of the PNMT gene (Egr-1, AP2, Ps 1, and MAZ) also contribute to the adrenergic phenotype of the human chromaffin cell [50]. Following an acute sympathetic response to stress, high plasma levels of noradrenaline and adrenaline provided by the sympathetic nervous system and adrenaline secreted by the medulla increase the metabolic rate of the body to produce

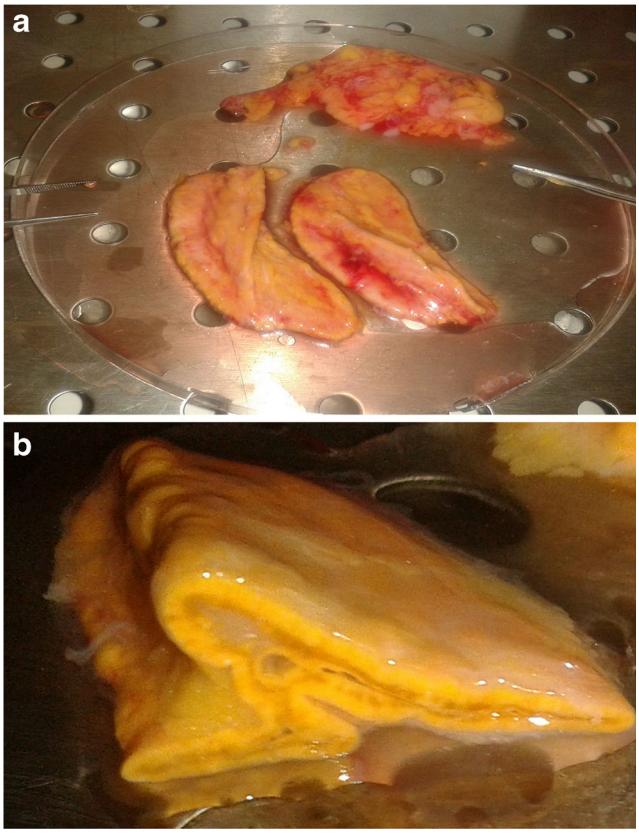

Fig. 1 Photographs of human adrenal glands obtained from organ donors. **a** Two whole human adrenal glands in which the surrounding fat has been removed. **b** Human adrenal gland sectioned. Note that under the capsule the cortex and medulla are intermingled and there is no separation between them

energy and adapt the cardiovascular system and musculature to defense or escape. Whenever sympathetic activity increases, the splanchnic nerve releases acetylcholine (ACh) that will bind to nicotinic acetylcholine receptors (nAChRs) and muscarinic receptors in the chromaffin cells of the adrenal gland.

nAChRs are ligand-gated cationic channels that mediate fast synaptic transmission. There are 16 different nAChR subunits, α1–7, α9, α10, β1-β4, δ, and ε in adult mammals and γ in embryonic muscle, that assemble into pentamers to form a variety of nAChR subtypes. As the α3β4* (asterisk indicates the possible presence of additional subunits) nAChR subtype is classically considered the ganglionic subtype, chromaffin cells are expected to mainly express this receptor as well. The initial studies on nAChRs in chromaffin cells were performed in bovine and rat species, and showed that this was indeed the case. However, controversy exits regarding the precise subunit composition of nAChRs in bovine and rat chromaffin cells. Bovine chromaffin cells express α3β4* nAChRs but it is unknown whether they also express subtypes with β2 subunits [7]. In rat chromaffin cells, a previous report suggested the presence of α3β4 nAChRs as well as a subtype(s) with β2 subunits [14]. The functional role of α7 nAChR subunits in chromaffin cells is less clear. The α7 gene was cloned [18] and cell surface expression of α7 nAChRs was determined by binding experiments using the α7 and α9* nAChR antagonist α-bungarotoxin (α-Bgtx) [38, 49] and by antibody detection [16]. In addition, partial or full α-Bgtx block of nAChR agonist-elicited currents, calcium signals, or secretion in bovine chromaffin or PC12 cells [5, 13, 27] has also been shown. However, the catecholamine release process [25, 26, 45, 47] was found to be insensitive to α-Bgtx. Evidence for the presence of α7 nAChRs has also been provided in rat chromaffin cells through α7 mRNA detection [11, 14, 32, 41], and in PC12 cells by affinity purification and immunoblotting of α-Bgtx binding receptors [15]. In rat chromaffin cells, however, inward currents elicited by nicotine pulses were insensitive to α-Bgtx and low doses of methyllycaconitine (MLA), demonstrating lack of functional α7 receptors [14].

Given the relevance of nAChRs to the release of catecholamines by adrenal gland chromaffin cells and sympathetic neurons, and the number of drugs acting on nAChRs that are clinically used, it was of interest to characterize these receptors in native human cells. Our first study on human chromaffin cell nAChRs was published in 2007 [33]. It showed that the achievement of stable electrophysiological recordings of nAChRs and nAChR-evoked exocytosis in the perforated-patch mode of the patch-clamp technique was possible in chromaffin cells obtained from the adrenal glands of organ donors. Afterwards, we performed a detailed characterization of nAChR subtypes in human chromaffin cells using α-conotoxins (α-CTxs). We also investigated the contribution of these receptors to the exocytosis that would lead to neurotransmitter release, and the effect of some drugs of particular relevance for nicotine addition on the membrane potential.

nAChRs in human chromaffin cells of adrenal glands obtained from organ donors: characterization, contribution to exocytosis and pharmacology

Chromaffin cells were collected from adrenal glands of organ donors with "encephalic" or "cardiac" death. In the "encephalic death," donor brain has suffered an irreversible loss of function. In these donors, the heart continued to beat, kept "alive" by means of vasoactive drugs and mechanical ventilation that guaranteed the perfusion of organs. In the "cardiac death" condition, the donor heart has stopped beating due to cardiac arrest, and the lack of blood circulation rapidly provoked the death of the brain. To be considered viable, other organs must be perfused through cannulation of the femoral vessels within 2 h of cardiac arrest. Donors are transferred to the hospital with mechanical ventilation, cardiocompression, and medication to assess donation. In both cases, donors are considered to be dead since resuscitation is not possible. After obtaining the permits of the family and a judge, organs to be

transplanted and adrenals were removed from the donor. Adrenal glands were then placed into a preservation liquid and transported to our laboratory, where we performed the isolation and culture of the chromaffin cells [21, 33]. Experiments to record nAChR currents were started 24–48 h after platting the cells to allow recovery from enzyme digestion [2].

Characterization of nAChRs in human chromaffin cells

We first sought to address the subunit composition of nAChRs in these cells, with special attention paid to the α7 receptor, which is crucial in mediating rapid synaptic transmission [1, 17, 48, 53]. α7 mRNA detection in human medulla was previously shown by Mousavi and colleagues in 2001 [32]. Thus, the probability that this receptor subtype was expressed in human chromaffin cells was high. We found that the nAChR antagonists α-Bgtx (1 μM) and MLA (10 nM) blocked the nicotinic currents elicited by ACh by 6 ± 1.7 and 7 ± 1.6%, in an irreversible and reversible manner, respectively. Choline (10 mM) pulses induced a biphasic current with an initial α7 component (5.5 ± 0.4 ms rise time, 8.5 ± 0.4 ms time constant decay time), which was blocked by α-Bgtx or MLA, followed by a slower non-α7 component. The α7 nAChR specific agonist PNU-282987 also elicited rapidly activated currents (7.1 ± 0.4 ms for 3 μM and 5.5 ± 0.4 ms for 30 μM) that were also rapidly inactivated (10 ± 0.9 ms for 3 μM, and 9.8 ± 1.8 ms for 30 μM). α7 nAChR positive allosteric modulators, such as 5-hydroxyindole (1 mM) and PNU-120596 (10 μM), potentiated nAChR currents that could be blocked by α-Bgtx. α7 nAChR currents could be clearly recorded in all human chromaffin cells tested [35]. It is interesting to note that in bovine adrenal gland slices, α-BgTx sensitive receptors are restricted to medullary areas adjacent to the adrenal cortex and are colocalized with PNMT. Also, α7 nAChR transcripts are localized exclusively in adrenergic cells [12]. The expression of PNMT [51] and the α7 nAChR subunit gene [9] are activated by glucocorticoids. These findings may explain why α7 nAChR currents could be recorded in human chromaffin cells in which 99% of cells are adrenergic, while in other laboratories, their presence could not be clearly established in non-human species with more of a mixture of adrenergic and non-adrenergic cells.

To characterize non-α7 nAChR subtypes expressed in chromaffin cells, α-Ctxs were used. These toxins are peptides isolated from the venom of marine cone snails and have been useful in developing peptide analogs that selectively target specific nAChR subtypes. The use of these peptides together with molecular biology tools allowed us to conclude that the predominant heteromeric nAChR subtype expressed by human chromaffin cells is α3β4* with a minor population of β2* nAChRs [21]. This conclusion was achieved by means of the use of the following peptides: LvIA(N9R,V10A) that targets human α3β2, α6/α3β2β3, and β3α6β2α4β2 nAChRs heterologously expressed in *Xenopus* oocytes with IC_{50} values of 3.3, 13.5, and 11.4 nM, respectively [21]; α-CTx BuIA(T5A,P6O) that targets α3β4 and α6/α3β4 nAChRs heterologously expressed in *Xenopus* oocytes with IC_{50} values of 166 and 7.4 nM, respectively [21]; and α-CTx PeIA(A7V,S90H,V10A,N11R,E14A, an α6β2 and α6β4 nAChR antagonist that targets $\alpha6_{M211L,cyt\alpha3}\beta4$, α6/α3β2β3, and β3α6β2α4β2 receptors heterologously expressed in *Xenopus* oocytes with IC_{50} values of 1.6, 3.8, and 6.3 nM, respectively [21]; and α-CTx ArIB(V11L,V16D), that targets α7 human native receptors [24], whereas human non-α7 nAChRs heterologously expressed in *Xenopus* oocytes are insensitive to this toxin [21].

nAChR currents elicited by ACh pulses in human chromaffin cells were blocked by 7 ± 2% with 100 nM LvIA(N9R,V10A), showing a minor contribution of β2 subunits in the nAChR composition. Currents were blocked by 98 ± 0.3% with 1 μM α-CTx BuIA(T5A,P6O) in the same cells, treated with 100 nM α-CTx ArIB(V11L,V16D) to block α7 nAChRs. In addition, α-CTx PeIA(A7V,S90H,V10A,N11R,E14A) only inhibited nicotinic currents in human chromaffin cells at concentrations of 100 nM or higher suggesting that there were few α6*-containing nAChRs present in these cells [21].

We performed molecular biology to confirm electrophysiological data. We assessed human adrenal gland tissue for the expression of nAChR subunit mRNAs using both end-point and quantitative real-time PCR (qPCR) methodologies. mRNAs for multiple nAChR subunits including α2, α3, α4, α5, α6, α7, α10, β2, and β4 subunits were detected. However, transcripts for α3, α7, and β4 subunits were found to be the most abundant subunits present. Transcripts for α5 and β2 were somewhat less abundant while those for α2, α6, and α10 were nearly absent. Transcripts for α4, α9, and β3 were detected infrequently. Internal controls were also performed by comparing the expression levels of α2, α3, α4, α5, α6, α7, α10, β2, and β4 subunits in adrenal medullary tissue to human brain. These experiments indicated that in adrenal gland, transcripts for α3 were more abundant compared to α6 whereas in human brain, α6 were more abundant than α3. We reassessed these results by performing the experiments on adrenal chromaffin cells isolated and cultured to avoid contamination of other cells. qPCR experiments in isolated chromaffin cells confirmed results obtained in adrenal medulla [21].

Contribution to exocytosis of nAChRs in human chromaffin cells

We recorded the plasma membrane capacitance increment in the voltage-clamp mode of the patch-clamp technique as an index of exocytosis to investigate the contribution of nAChR currents to the exocytotic process. However, ACh evokes action potentials that depolarize the cell and activate voltage-dependent calcium channels (VDCC). Therefore, to evaluate the overall exocytosis evoked by ACh, it is necessary to apply a protocol that allows recording of capacitance increments elicited under non-voltage-clamped conditions. To achieve this condition, we developed a "triple-step" protocol to measure plasma membrane capacitance increments due to changes in the membrane potential elicited by the nicotinic agonist. In this way, the plasma membrane capacitance that can be only measured under the whole-cell configuration is recorded before and after a pulse of ACh applied in the current-clamp configuration. By performing this protocol, we found that the contribution to the exocytosis of calcium entry through the nAChR ionophore may be of similar magnitude to that achieved by calcium entry through VDCC due to depolarization [33]. Using this protocol, we have been able to observe that α7 nAChR currents did not evoke exocytosis by themselves, but the depolarization provoked by these currents was able to elicit exocytosis [35]. On the other hand, current flowing through the α3β4* nAChR ionophore is able to elicit exocytosis by itself or by evoking depolarization [33, 36].

Use of α-Ctxs: some considerations

The fact that α-CTxs selective for rat α6*-containing nAChRs expressed in heterologous systems inhibited human chromaffin cell nAChRs with similar IC_{50} values led us to initially conclude that the predominant nAChR expressed in human chromaffin cells was the α6β4* subtype [36]. In this previous study, we used the mutant analog of α-Ctx MII, the α-Ctx MII[H9A,L15A] (α-Ctx MII[H9A,L15A]) which primarily targets rat α6* nAChRs with respect to rat α3 nAChRs expressed heterologouly in *Xenopus* oocytes [30] and exhibited a lower IC_{50} for α6β2* (2.4 nM) with respect to α6β4* nAChRs (269 nM). The IC_{50} value obtained for this toxin in human chromaffin cells was 217.8 nM [36], similar to the data previously reported for rat α6β4* nAChRs expressed in *Xenopus* oocytes. For that reason, the conclusion was that an α6β4* nAChRs was the predominant nAChR subtype expressed in these cells.

Later on some reports showed evidences on the differential effect of α-Ctxs on rat versus human nAChRs expressed in heterologous systems [3, 52], which was further confirmed in our lab [21]. In addition, we found some inconsistencies between α-Ctx MII[H9A, L15A] IC_{50} values for inhibition of human adrenal chromaffin cell nAChRs (217.8 nM) [36] and human α6/α3β4* nAChRs heterologously expressed in *Xenopus* oocytes (13.3 nM) [19]. Thus, species differences are a key factor to be considered when characterizing nAChR subtypes.

The effects of α-Ctxs on human nAChRs expressed in *Xenopus* oocytes and chromaffin cells are summarized in Table 1. A comparison between the effects of α-Ctxs on rat and human α3β4 and α6β4 subtypes is summarized in Table 2.

Pharmacology of nAChRs in human chromaffin cells

Tobacco smoking is the major cause of cardiovascular morbidity and mortality, and therefore, quitting smoking is crucial, especially for patients with some cardiovascular disease. However, some drugs used in the cessation smoking therapy are central nervous system nAChR agonists and may be also acting on ganglionic α3β4 nAChRs. This is the case of varenicline, a drug clinically used for the treatment of nicotine addiction. Varenicline activates heterologously expressed nAChRs of the α4β2 [31, 39], α6β2 [8], α3β4 [6, 31, 40, 43, 46], and α7 subtypes [31].

A case-report of varenicline-triggered pheochromocytoma crisis in a smoking subject suggested that varenicline might have activity towards adrenal gland chromaffin cell nAChRs [23]. In addition, some evidences regarding possible cardiovascular adverse effects of varenicline have been reported [37, 42]. However, information concerning the activity of varenicline on native human α3β4 nAChRs was not available. Thus, we evaluated the action of varenicline alone and in the presence of nicotine on nAChRs in human chromaffin cells, as well as on the excitability of these cells.

We performed patch-clamp experiments under the current-clamp configuration to evaluate the effects of varenicline on the plasma membrane excitability elicited by native α3β4* nAChRs in human chromaffin cells and compared them to those of nicotine. Varenicline and nicotine activated α3β4* nAChRs with EC_{50} values of 1.8 (1.2–2.7) μM and 19.4 (11.1–33.9) μM, respectively. Perfusion of therapeutically relevant doses of varenicline (50 or 100 nM) showed very little effect on action potential firing evoked by 10 ms ACh in the current-clamp mode. However, perfusion of 250 nM varenicline increased the number of action potentials fired by 436 ± 150% compared to control conditions. In contrast, nicotine showed no effect on action potential firing at any of the concentrations tested (50, 100, 250, and 500 nM). However, the presence of nicotine may potentiate the effects of varenicline. In smoking cessation therapy with varenicline, smokers should establish a date to stop smoking and treatment with varenicline should start 1 to 2 weeks before this date. In addition, nicotine replacement therapy is sometimes combined with varenicline to

Table 1 IC50 values for inhibition of human nAChRs expressed in *Xenopus* oocytes and adrenal chromaffin cell nAChRs. Values in parentheses are 95% confidence intervals

	MII (S4A,E11A,L15A)	MII[H9A,L15A]	BuIA(T5A,P60)	LvIA(N9R,V10A)	PeIA(A7V,S9H,V10A,N11R,E14A)	AuIB	TxID
α7							>30 μM [d]
α3β2		~ 10 μM [b]	>10 μM [b,c]	3.3 (2.4–4.7) nM [c]	6.1 (3.6–10.3) μM [c]		
α3β4		1.4 (1.1–1.7) μM [c]	166 (141–196) nM [c]	>10 μM [c]	3.7 (2.4–5.8) μM [c]	>10 μM [c]	8.7 (7.8–9.7) nM [d]
β4α3β4α3α5(D)			147 (125–173) nM [c]	>10 μM [c]	9.2 (6.4–13.4) μM [c]		
α4β2			>10 μM [b,c]	195 (133–284) nM [c]	>10 μM [c]		
α4β4			>10 μM [b,c]	>10 μM [c]	>10 μM [c]		
α6/α3β2β3			>10 μM [b,c]	13.5 (8.6–21.2) nM [c]	3.8 (3.2–4.5) nM [c]		
β3α6β2α4β2			>10 μM [c]	11.4 (8.1–16.0) nM [c]	6.3 (5.6–7.1) nM [c]		
α6/α3β4		13.3 (9.7–18.1) nM [b]	7.4 (6.5–8.3) nM [c]	1.0 (7.5–13.3) μM [c]	N.D.	360 (305–424) nM [c]	
			11.1 (9.1–13.6) nM [b]				
$\alpha6_{M211L,cyt\alpha3}/\beta4$			11.3 (10.1–12.7) nM [c]	2.8 (2.5–3.3) μM [c]	1.6 (1.2–2.2) nM [c]		
ACC	33 nM [a]	217.8 nM [a]	46.7 (39.8–55.1) nM [c]	>1 μM [c]	>1 μM [c]		24.1 (20.1–28.5) nM [d]

ACC Adrenal chromaffin cells

[a] Pérez-Alvarez et al. [36]

[b] Hernández-Vivanco et al. [19]

[c] Hone et al. [21]

[d] Hone et al. [22]

Table 2 Comparison of α-Ctx IC50 values for inhibition of rat versus human nAChRs expressed in *Xenopus* oocytes (taken from Hone et al. [22] with some modifications)

	rα3β4	hα3β4	rα6β4	hα6β4
BuIA(T5A,P60)	1.2 μM[a]	166 nM[d]	58 nM[a]	7 nM[d]
MII[H9A,L15A]	7.8 μM[a]	1.4 μM[b]	269 nM[a]	13 nM[b]
PeIA(A7V,S9H,V10A, N11R,E14A)	> 10 μM[c]	3.7 μM[d]	44 nM[c]	1.6 nM[d]
AuIB	750 nM[e]	> 10 μM[d]	7.3 μM[f]	360 nM[d]
TxID	12.5 nM[h]	8.7 nM[g]	96 nM[h]	

[a] Azam et al. [4]

[b] Hernández-Vivanco et al. [19]

[c] Hone et al. [20]

[d] Hone et al. [21]

[e] Luo et al. [28]

[f] Smith et al. [44]

[g] Hone et al. [22]

[h] Luo et al. [29]

improve quit rates [10]. To examine this, we tested 50 nM nicotine together with 100 nM varenicline and obtained an increase of the action potential firing by 290 ± 104%. These results demonstrate that therapeutic concentrations of varenicline alone are unlikely to alter the adrenal chromaffin cell's behavior and response to ACh, but in combination with nicotine, varenicline increases action potential firing, which may lead to an increase in neurotransmitter release [21].

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References

1. Alkondon M, Pereira EF, Albuquerque EX (1998) Alpha-Bungarotoxin- and methyllycaconitine-sensitive nicotinic receptors mediate fast synaptic transmission in interneurons of rat hippocampal slices. Brain Res 810:257–263
2. Almazan G, Aunis D, García AG, Montiel C, Nicolás GP, Sáncez-Gacía P (1984) Effects of collagenase on the release of [3H]-noradrenaline from bovine cultured adrenal chromaffin cells. Br J Pharmacol 81:599–561
3. Azam L, McIntosh JM (2012) Molecular basis for the differential sensitivity of rat and human α9α10 nAChRs to α-conotoxin RgIA. J Neurochem 122:1137–1144

4. Azam L, Maskos U, Changeux JP, Dowell CD, Christensen S, De Biasi M, McIntosh JM (2010) α-Conotoxin BuIA[T5A;P6O]: a novel ligand that discriminates between α6ß4 and α6ß2 nicotinic acetylcholine receptors and blocks nicotine-stimulated norepinephrine release. FASEB J 24:5113–5123
5. Blumenthal EM, Conroy WG, Romano SJ, Kassner PD, Berg DK (1997) Detection of functional nicotinic receptors blocked by alpha-bungarotoxin on PC12 cells and dependence of their expression on post-translational events. J Neurosci 17:6094–6104
6. Campling BG, Kuryatov A, Lindstrom J (2013) Acute activation, desensitization and smoldering activation of human acetylcholine receptors. PLoS One 8:e79653
7. Campos-Caro A, Smillie FI, Dominguez del Toro E, Rovira JC, Vicente-Agullo F, Chapuli J, Juiz JM, Sala S, Sala F, Ballesta JJ, Criado M (1997) Neuronal nicotinic acetylcholine receptors on bovine chromaffin cells: cloning, expression, and genomic organization of receptor subunits. J Neurochem 68:488–497
8. Capelli AM, Castelletti L, Chen YH et al (2011) Stable expression and functional characterization of a human nicotinic acetylcholine receptor with alpha6beta2 properties: discovery of selective antagonists. Br J Pharmacol 163:313–329
9. Carrasco-Serrano C, Criado M (2004) Glucocorticoid activation of the neuronal nicotinic acetylcholine receptor α7 subunit gene: involvemente of transcripticon factor Egr-1. FEBS Lett 566:247–250
10. Chang PH, Chiang CH, Ho WC, Wu PZ, Tsai JS, Guo FR (2015) Combination therapy of varenicline with nicotine replacement therapy is better than varenicline alone: a systematic review and meta-analysis of randomized controlled trials. BMC Public Health 15: 689
11. Colomer C, Olivos-Ore LA, Vincent A, McIntosh JM, Artalejo AR, Guerineau NC (2010) Functional characterization of alpha9-containing cholinergic nicotinic receptors in the rat adrenal medulla: implication in stress-induced functional plasticity. J Neurosci 30: 6732–6742
12. Criado M, Domínguez del Toro E, Carrasco-Serrano C, Smillie FI, Juíz JM, Viniegra S, Ballesta JJ (1997) Differential expression of alpha-bungarotoxin-sensitive neuronal nicotinic receptors in adrenergic chromaffin cells: a role for transcription factor Egr-1. J Neurosci 17:6554–6564
13. Del Barrio L, Egea J, Leon R, Romero A, Ruiz A, Montero M, Alvarez J, López MG (2011) Calcium signalling mediated through alpha7 and non-alpha7 nAChR stimulation is differentially regulated in bovine chromaffin cells to induce catecholamine release. Br J Pharmacol 162:94–110
14. Di Angelantonio S, Matteoni C, Fabbretti E, Nistri A (2003) Molecular biology and electrophysiology of neuronal nicotinic receptors of rat chromaffin cells. Eur J Neurosci 17:2313–2322
15. Drisdel RC, Green WN (2000) Neuronal alpha-bungarotoxin receptors are alpha7 subunit homomers. J Neurosci 20:133–139
16. El-Hajj RA, McKay SB, McKay DB (2007) Pharmacological and immunological identification of native alpha7 nicotinic receptors: evidence for homomeric and heteromeric alpha7 receptors. Life Sci 81:1317–1322
17. Frazier CJ, Buhler AV, Weiner JL, Dunwiddie TV (1998) Synaptic potentials mediated via alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons. J Neurosci 18: 8228–8235
18. García-Guzmán M, Sala F, Sala S, Campos-Caro A, Stuhmer W, Gutierrez LM, Criado M (1995) alpha-Bungarotoxin-sensitive nicotinic receptors on bovine chromaffin cells: molecular cloning, functional expression and alternative splicing of the alpha 7 subunit. Eur J Neurosci 7:647–655
19. Hernandez-Vivanco A, Hone AJ, Scadden M, Carmona Hidalgo B, McIntosh JM, Albillos A (2014) Monkey adrenal chromaffin cells express α6β4* nicotinic acetylcholine receptors. PLoS One 9(4): e94142
20. Hone AJ, Ruiz M, Scadden M, Christensen S, Gajewiak J, Azam L, McIntosh JM (2013) Positional scanning mutagenesis of α-conotoxin PeIA identifies critical residues that confer potency and selectivity for α6/α3β2β3 and α3β2 nicotinic acetylcholine receptors. J Biol Chem 288:25428–25439
21. Hone AJ, McIntosh JM, Azam L, Lindstrom J, Lucero L, Whiteaker P, Passas J, Blazquez J, Albillos A (2015) α-Conotoxins identify the α3β4 subtype as the predominant nicotinic acetylcholine receptor expressed in human adrenal chromaffin cells. Mol Pharmacol 88:881–893
22. Hone AJ, McIntosh JM, Rueda-Ruzafa L, Passas J, De Castro-Guerín C, Blazquez J, González-Enguita C, Lindstrom J, Albillos A (2017) Therapeutic concentrations of varenicline and nicotine increase action potential firing in human adrenal chromaffin cells. J Neuroch 140:37–52
23. Hukkanen J, Ukkola O, Benowitz NL (2010) Varenicline and pheochromocytoma. Ann Intern Med 152:335–336
24. Innocent N, Livingstone PD, Hone A, Kimura A, Young T, Whiteaker P, McIntosh JM, Wonnacott S (2008) Alpha-conotoxin Arenatus IB[V11L,V16D] [corrected] is a potent and selective antagonist at rat and human native alpha7 nicotinic acetylcholine receptors. J Pharmacol Exp Ther 327:529–537
25. Kilpatrick DL, Slepetis R, Kirshner N (1981) Inhibition of catecholamine secretion from adrenal medulla cells by neurotoxins and cholinergic antagonists. J Neurochem 37:125–131
26. Kumakura K, Karoum F, Guidotti A, Costa E (1980) Modulation of nicotinic receptors by opiate receptor agonists in cultured adrenal chromaffin cells. Nature 283:489–492
27. López MG, Montiel C, Herrero CJ, García-Palomero E, Mayorgas I, Hernandez-Guijo JM, Villarroya M, Olivares R, Gandia L, McIntosh JM, Olivera BM, García AG (1998) Unmasking the functions of the chromaffin cell alpha7 nicotinic receptor by using short pulses of acetylcholine and selective blockers. Proc Natl Acad Sci U S A 95:14184–14189
28. Luo S, Kulak JM, Cartier GE, Jacobsen RB, Yoshikami D, Olivera BM, McIntosh JM (1998) alpha-conotoxin AuIB selectively blocks alpha3 beta4 nicotinic acetylcholine receptors and nicotine-evoked norepinephrine release. J Neurosci 18:8571–8579
29. Luo S, Zhangsun D, Wu Y, Zhu X, Hu Y, McIntyre M, Christensen S, Akcan M, Craik DJ, McIntosh JM (2013) Characterization of a novel a-conotoxin from conus textile that selectively targets a6/a3b2b3 nicotinic acetylcholine receptors. J Biol Chem 288:894–902
30. McIntosh JM, Azam L, Staheli S, Dowell C, Lindstrom JM, Kuryatov A, Garrett JE, Marks MJ, Whiteaker P (2004) Analogs of alpha-conotoxin MII are selective for alpha6-containing nicotinic acetylcholine receptors. Mol Pharmacol 65:944–952
31. Mihalak KB, Carroll FI, Luetje CW (2006) Varenicline is a partial agonist at alpha4beta2 and a full agonist at alpha7 neuronal nicotinic receptors. Mol Pharmacol 70:801–805
32. Mousavi M, Hellstrom-Lindahl E, Guan ZZ, Bednar I, Nordberg A (2001) Expression of nicotinic acetylcholine receptors in human and rat adrenal medulla. Life Sci 70:577–590
33. Pérez-Alvarez A, Albillos A (2007) Key role of the nicotinic receptor in neurotransmitter exocytosis in human chromaffin cells. J Neurochem 103:2281–2290
34. Pérez-Alvarez A, Hernandez-Vivanco A, Cano-Abad M, Albillos A (2008) Pharmacological and biophysical properties of Ca^{2+} channels and subtype distributions in human adrenal chromaffin cells. Pflugers Arch 456:1149–1162
35. Pérez-Alvarez A, Hernandez-Vivanco A, Gregorio SA, Tabernero A, McIntosh JM, AlbillosA (2012a) Pharmacological characterization of native α7 nAChRs and their contribution to depolarization-

elicited exocytosis in human chromaffin cells. Br J Pharmacol 165: 908–921
36. Pérez-Alvarez A, Hernandez-Vivanco A, McIntosh JM, Albillos A (2012b) Native α6β4* nicotinic receptors control exocytosis in human chromaffin cells of the adrenal gland. FASEB J 26:346–354
37. Prochaska JJ, Hilton JF (2012) Risk of cardiovascular serious adverse events associated with varenicline use for tobacco cessation: systematic review and meta-analysis. BMJ 344:e2856
38. Quik M, Geertsen S, Trifaró JM (1987) Marked up-regulation of the beta-bungarotoxin site in adrenal chromaffin cells by specific nicotinic antagonists. Mol Pharmacol 31:385–391
39. Rollema H, Chambers LK, Coe JW et al (2007) Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. Neuropharmacology 52:985–994
40. Rollema H, Russ C, Lee TC, Hurst RS, Bertrand D (2014) Functional interactions of varenicline and nicotine with nAChR subtypes implicated in cardiovascular control. Nicotine Tob Res 16:733–742
41. Rust G, Burgunder JM, Lauterburg TE, Cachelin AB (1994) Expression of neuronal nicotinic acetylcholine receptor subunit genes in the rat autonomic nervous system. Eur J Neurosci 6: 478–485
42. Singh S, Loke YK, Spangler JG, Furberg CD (2011) Risk of serious adverse cardiovascular events associated with varenicline: a systematic review and meta-analysis. CMAJ 183:1359–1366
43. Stokes C, Papke RL (2012) Use of an alpha3beta4 nicotinic acetylcholine receptor subunit concatamer to characterize ganglionic receptor subtypes with specific subunit composition reveals species-specific pharmacologic properties. Neuropharmacology 63:538–546
44. Smith NJ, Hone AJ, Memon T, Bossi S, Smith TE, McIntosh JM, Olivera BM, Teichert RW (2013) Comparative functional expression of nAChR subtypes in rodent DRG neurons. Front Cell Neurosci 7:225
45. Tachikawa E, Mizuma K, Kudo K, Kashimoto T, Yamato S, Ohta S (2001) Characterization of the functional subunit combination of nicotinic acetylcholine receptors in bovine adrenal chromaffin cells. Neurosci Lett 312:161–164
46. Tammimaki A, Herder P, Li P, Esch C, Laughlin JR, Akk G, Stitzel JA (2012) Impact of human D398N single nucleotide polymorphism on intracellular calcium response mediated by alpha3beta4alpha5 nicotinic acetylcholine receptors. Neuropharmacology 63:1002–1011
47. Trifaró JM, Lee RW (1980) Morphological characteristics and stimulus-secretion coupling in bovine adcrenal chromaffin cell cultures. Neuroscience 5:1533–1546
48. Ullian EM, McIntosh JM, Sargent PB (1997) Rapid synaptic transmission in the avian ciliary ganglion is mediated by two distinct classes of nicotinic receptors. J Neurosci 17:7210–7219
49. Wilson SP, Kirshner N (1977) The acetylcholine receptor of the adrenal medulla. J Neurochem 28:687–695
50. Wong DL (2003) Why is the adrenal adrenergic? Endocr Pathol 14: 25–36
51. Wurtman RJ, Axelrod J (1965) Adrenaline synthesis: control by the pituitary gland and adrenal glucocorticoids. Science 150:1464–1465
52. Yu R, Kompella SN, Adams DJ, Craik DJ, Kaas Q (2013) Determination of the α-conotoxin Vc1.1 binding site on the α9α10 nicotinic acetylcholine receptor. J Med Chem 56: 3557–3567
53. Zhang ZW, Coggan JS, Berg DK (1996) Synaptic currents generated by neuronal acetylcholine receptors sensitive to alpha-bungarotoxin. Neuron 17:1231–1240