© *Pharmacology & Toxicology* 2003, **93**, 169–173.
Printed in Denmark . All rights reserved

Pharmacology & Toxicology
ISSN 0901-9928

Nicotinic Acetylcholinergic Receptors Regulate the Intraspinal Release of Acetylcholine in Male Rats

Mahinda Kommalage and A. Urban Höglund

Department of Neuroscience, Division of Comparative Medicine, Uppsala University, Biomedical Centre, Box 572, S-751 23 Uppsala, Sweden

(Received March 11, 2003; Accepted June 26, 2003)

Abstract: Activation of cholinergic receptors in the spinal cord increases the intraspinal release of acetylcholine (ACh) and produces potent analgesia. The mechanisms that regulate the release of spinal ACh are not fully known. In the present study, we investigated the role of nicotinic ACh receptors in the regulation of intraspinal ACh release. Using an *in vivo* intraspinal microdialysis technique, nicotine was administered alone and in combination with the nicotinic antagonists mecamylamine (50 μM), dihydro-β-erythroidine (DβE) (500 μM) and methyllycaconitine (MLA) (40 nM). Administration of nicotine (1 μM–1 mM) produced a dose dependent increase of intraspinal ACh release, while 10 mM nicotine resulted in dramatic increase in ACh release followed by a decrease to baseline. Administration of mecamylamine or DβE also induced an increased ACh release while MLA caused a decreased release. Mecamylamine and DβE, but not MLA pretreatment attenuated the stimulatory effect of 100 μM nicotine on intraspinal ACh release. It is suggested that spinal ACh release is regulated by different nicotinic ACh receptors. These receptors may tonically regulate spinal ACh release either directly or indirectly via inhibitory interneurones. Some of these receptors may be desensitised by high nicotine concentrations leading to a reduction of ACh release.

Activation of cholinergic receptors in the spinal cord with both muscarinic agonists such as oxotremorine and carbachol as well as nicotinic agonists such as nicotine and epibatidine produces potent antinociception (Christensen & Smith 1990; Naguib & Yaksh 1997; Khan *et al.* 1998; Machelska *et al.* 1999). Several mechanisms have been suggested for cholinomimetic-induced antinociception including reduced activity in secondary nociceptive fibres (Jurna *et al.* 1993), decreased release of primary nociceptive fibre transmitters such as substance P (Smith *et al.* 1989) and glutamate (Li & Zhuo 2001), and modulation of release of transmitters in descending pain inhibitory pathways (Meyer *et al.* 2000; Rogers & Iwamoto 1993).

Recently, it was shown that both oxotremorine and epibatidine increase the intraspinal release of acetylcholine (ACh) (Höglund *et al.* 2000). This finding is consistent with the observation that intrathecal administration of the cholinesterase inhibitor neostigmine produces antinociception (Bouaziz *et al.* 1995; Hama *et al.* 2001). This finding is however paradoxical since it would mean that the release of ACh induces an even further release of the transmitter itself, but this is not the case. As determined by microdialysis, the intraspinal ACh release is stable for long periods when Ringer's solution containing neostigmine is administered (Höglund *et al.* 2000). When oxotremorine or epibatidine is administered, ACh release is increased but the release is soon stabilised at a higher level. These observations indicate that intraspinal ACh release from interneurones or from descending pathways (Bouaziz *et al.* 1996; Cordero-Erausquin & Changeux 2001) is tonically regulated, possibly by some of the other transmitters known to be present in the spinal dorsal horn e. g. norepinephrine and serotonin, which are released from descending pain-transmission inhibitory pathways (Fürst 1999).

Acetylcholine may have an important role in the regulation of the spinal cord pain threshold since not only administration of oxotremorine and epibatidine (Höglund *et al.* 2000), but also potent analgesics such as morphine (Bouaziz *et al.* 1996) and clonidine (Klimscha *et al.* 1997), increase intraspinal ACh release. This suggestion is strengthened by the fact that administration of the cholinergic antagonists, atropine or mecamylamine, or the choline uptake inhibitor, hemicolinium-3 attenuates the analgesic action of morphine (Chen & Pan 2001). It is therefore of importance to study which mechanisms regulate the intraspinal release of ACh. Previously, we have shown that activation of spinal muscarinic receptors increases the intraspinal release of ACh and that inhibition of these receptors decreases ACh release (Höglund *et al.* 2000). In the present study, we investigated the regulatory role of intraspinal nicotinic acetylcholinergic receptors (nAChRs).

The presence of nAChRs has been demonstrated in both ascending nociceptive pathways and descending pain modulatory pathways including the spinal dorsal horn (Roberts *et al.* 1995), nucleus raphe magnus (Bitner *et al.* 1998; Iwamoto 1991), periaqueductal grey matter (Adem *et al.* 1989), pedunculopontine tegmental nucleus (Iwamoto

Author for correspondence: A. Urban Höglund, Department of Neuroscience, Division of Comparative Medicine, Uppsala University, BMC, Box 572, S-751 23 Uppsala, Sweden (fax +46 18 50 17 40, e-mail Urban.Hoglund@BMC.uu.se).

1991), and thalamus (Ryan & Loiacono 2000). It is not yet clear which subunit combinations are of relevance for modulation of nociception. A primary site for the analgesic action of nicotinic agonists also remains to be defined. Administration of nicotine, epibatidine, or epibatidine derivatives systemically or by microinjection into the nucleus raphe magnus and the pedunculopontine tegmental nucleus produces antinociception in animals (Iwamoto 1991; Decker *et al.* 1998). In the spinal cord, nicotinic agonists can produce both antinociceptive and pronociceptive effects (Khan *et al.* 1998 & 2001). The different actions of nicotinic agonists in the spinal cord and in the brain have been proposed to be related to expression of different nAChRs in these tissues (Khan *et al.* 1994).

In the present study the effect of nicotine and nAChR antagonists on intraspinal ACh release was studied using our previously described *in vivo* intraspinal microdialysis technique (Höglund *et al.* 2000). The antagonists mecamylamine, dihydro-β-erythroidine (DβE) and methyllycaconitine (MLA), which have different affinities and modes of action on nAChRs, were used both alone and in combination with nicotine.

Nicotine, mecamylamine and DβE administration increased the intraspinal release of ACh whereas MLA decreased ACh release. Mecamylamine and DβE pretreatments were found to attenuate the effect of nicotine on intraspinal release of ACh. Administration of 10 mM nicotine initially caused a dramatic increase of spinal ACh release followed by a reduction to the basal level.

Materials and Methods

Rats. Male outbred Sprague-Dawley rats (B&K Universal, Sollentuna, Sweden) weighing 330 g–380 g were provided with free access to food (R36, Ewos, Vadstena, Sweden) and tap water at all times. The animals were kept on a 12 hr light/dark cycle one week before use in transparent polycarbonate cages (L590×W385×H200 mm) with aspen wood shavings as bedding material. Room temperature was 21±1° and relative humidity was between 40 – 60%. All experiments were approved by the animal ethics committee in Uppsala, Sweden.

Drugs and chemicals. Neostigmine bromide, acetylcholine chloride, choline, nicotine, mecamylamine, dihydro-β-erythroidine (DβE), and methyllycaconitine (MLA) were purchased from Sigma Sweden AB (Stockholm, Sweden). The salts NaCl, $CaCl_2$, KCl, Na_2HPO_4, were purchased from Kebo lab (Spånga, Sweden).

Microdialysis. Anaesthesia was induced with 4.5% isoflurane (Abbott Scandinavia AB, Solna, Sweden) in 100% oxygen. Intubated rats were connected to a Harvard ventilator (Harvard Apparatus inc., South Natick, MA, USA) and placed on a heated pad to maintain perirectal temperature at 37.5°. Anaesthesia was continued with 2.5–3.5% isoflurane during surgery and at 1.5% during microdialysis. The end-tidal pCO_2 was at all times kept at 4 kPa.

For insertion of the microdialysis probe (Marsil Scientific, San Diego, CA, USA) a midline incision was made at the back of the skull (Marsala *et al.* 1995). The neck muscles were removed carefully and the *dura* and *pia mater* were cut at *cisterna magna.* The spinal microdialysis probe was inserted intraspinally so that the tip was at about the C5 level dorsally. The probe was constructed from a hollow fibre with 300 μm outer diameter that has a cut-off at 11 kDa molecular weight. The dialysis membrane was bent to form a U-shaped loop with a length of 12 mm. After insertion of the microdialysis probe, the rats rested for 40 min. before the spinal microdialysis was commenced. The probe was perfused with Ringer's solution (147 mM NaCl, 2.4 mM $CaCl_2$, 4.0 mM KCl) containing 10 μM neostigmine to prevent degradation of ACh at a flow rate of 2.5 μl/min. ACh was quantified on line by HPLC as described previously (Höglund *et al.* 2000). The use of neostigmine is an experimental necessity to enable HPLC detection of ACh and the administration of neostigmine does not influence drug-receptor studies (Damsma *et al.* 1987 & 1988), neither in brain where the muscarinic M2 receptor function as an autoreceptor (Billard *et al.* 1995; Moor *et al.* 1995; Roth *et al.* 1996) nor in the spinal cord where the ACh release is suggested not to be autoreceptor regulated (Höglund *et al.* 2000).

In each experiment *in vitro* pre- and post-recovery of the probes were assessed by dialysis of a 10 pmol standard to make sure that the probes were not damaged during the experiment.

Doses of drugs. Before administration of drugs, the basal release of ACh was determined by collecting five 10 min. cycles with Ringer's solution. The drugs were dissolved in Ringer's solution and administered via the dialysis probe using a syringe pump with 2.5 μl/min. flow rate. To measure the effect of nicotine on ACh release, nicotine was used in a dose range from 1 μM to 10 mM. The antagonists mecamylamine (50 μM), DβE (500 μM) and MLA (40 nM) were used to investigate the influence of an antagonism of nAChRs on ACh release. 100 μM nicotine was used for administration after mecamylamine, DβE or MLA. These concentrations were based on previous experiments on spinal cord (Khan *et al.* 1994; Cordero-Erausquin & Changeux 2001).

As we used a microdialysis probe for drug administration, tissue drug concentration would be lower than the dialysed concentrations. We found in *in vitro* experiments, in which ACh was administered via the probe, that the concentration of ACh in an external volume of 30 μl gradually increased and was stabilised at 45% of the dialysed concentration, 50 min. after start of administration. This *in vitro* experiment indicates that small molecules such as nicotine and nicotinic antagonists used in the present experiments maximally can reach 45% of the dialysed concentrations. In a previous microdialysis experiment, in which a higher perfusion rate was used (5 μl/min.) Lawand *et al.* (1999) estimated the transfer of mecamylamine out of the dialysis fibre to <15%. This estimate corresponds with our findings. Depending on fat solubility and other factors influencing distribution, the tissue concentration of the dialysed drugs may be lower than the concentration in the dialysis probe.

Statistics. All statistical analyses were performed using SPSS version 10.0.5 (SPSS Inc., Chicago, Illinois, USA). The effect of the various substances was expressed as percent change from baseline

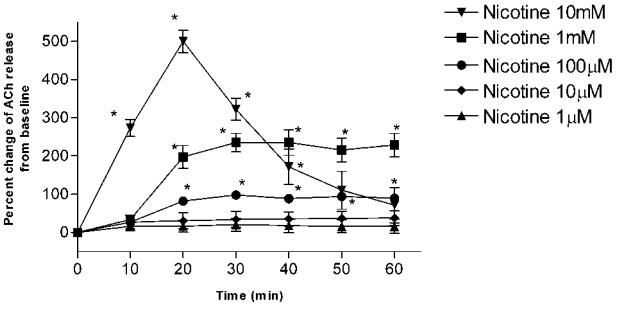

Fig. 1. Effects of microdialysis administration of 1μM (number of experiments=4), 10 μM (n=5), 100 μM (n=6), 1 mM (n=4) and 10 mM (n=4) concentrations of nicotine on the intraspinal release of acetylcholine (ACh). A * represents $P<0.05$ according to one-way ANOVA with Dunnett's post-hoc test versus basal ACh release.

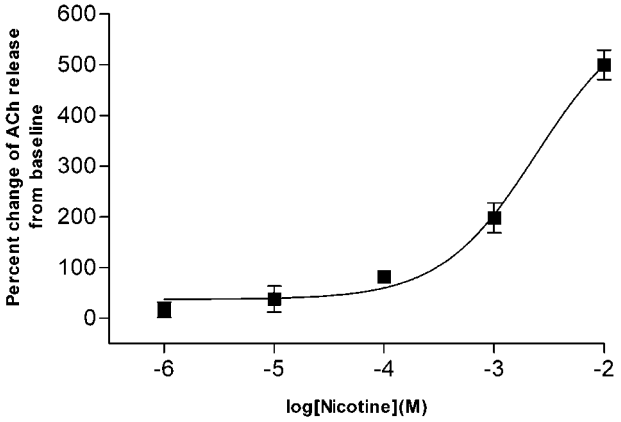

Fig. 2. A curve-fit analysis of the effect on intraspinal ACh release, measured 20 min. after start of administration, of the various nicotine concentrations administered intraspinally. Goodness of fit (R^2) was 0.99 and EC_{50}=1.7 mM.

defined as the mean release of ACh during five 10 min. sampling periods during which the microdialysis probe was perfused with Ringer's solution only. Analysis of variance with Dunnett's post-hoc test was used to calculate the statistical significance of effects of individual substances, against baseline release of ACh. Repeated measures analysis was used to calculate the statistical difference of intraspinal ACh release between substances and between substances and controls.

Results

Nicotine (1 μM–1 mM) microdialysis caused a dose dependent increase in intraspinal ACh release. Administration of 10 mM nicotine caused an initial increase of the ACh release followed by a reduction to the basal level (fig. 1). The effect of nicotine on ACh release was dose dependent with ED_{50}=1.7 mM (fig. 2).

Administration of the non-selective non-competitive nicotinic antagonist mecamylamine as well as the presumptively $\alpha_4\beta_2$ nAChRs selective, competitive antagonist DβE also resulted in an increased ACh release (fig. 3). Mecamylamine induced on average a 143% increase of ACh release from baseline between 50–70 min. after start of administration. DβE induced on average a 139% increase in ACh release from baseline between 50–70 min. after start of administration.

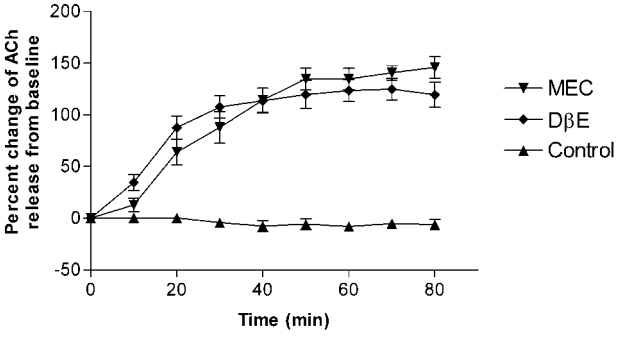

Fig. 3. Effects of 50 μM mecamylamine (MEC, n=6) and 500 μM dihydro-β-erythroidine (DβE, n=6) on the intraspinal ACh release in comparison to dialysis with Ringer's solution (Control, n=5). The effect of MEC and DβE was significantly different (P<0.05) from control administration of Ringer's solution, according to repeated measures analysis.

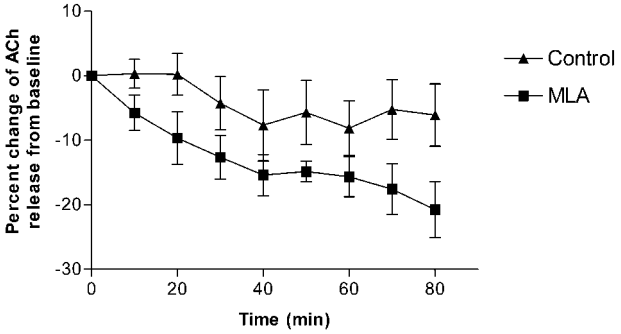

Fig. 4. Effects of 40 nM methyllycaconitine (MLA, n=5) on the intraspinal ACh release in comparison to dialysis with Ringer's solution (Control, n=5). The effect of MLA was significantly different (P<0.05) from control administration of Ringer's solution, according to repeated measures analysis.

The specific nicotinic α7 receptor antagonist, MLA produced a 16% decrease in ACh release from baseline between 50–70 min. after start of administration (fig. 4). Different concentrations of MLA were tried. The 40 nM concentration was found to produce the maximal reduction in ACh release, and was later used to antagonize the effect of nicotine. Higher doses such as 1μM resulted in a small increase in ACh release (data not shown).

Pre-treatment with either 50 μM mecamylamine or 500 μM DβE attenuated the effect of 100 μM nicotine (fig. 5). MLA (40 nM) pretreatment did not inhibit the ACh release stimulatory effect of 100 μM nicotine.

Discussion

The present data confirm that the net effect of nicotinic agonists administered via microdialysis to the spinal cord is

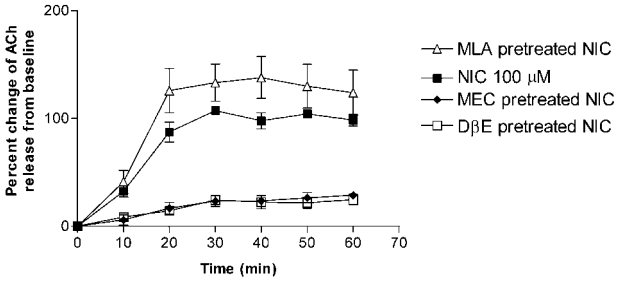

Fig. 5. Effects of 100 μM nicotine on intraspinal ACh release after 50 μM mecamylamine (MEC, n=4), 500 μM dihydro-β-erythroidine (DβE, n=8), 40 nM methyllycaconitine (MLA, n=4) pre-treatment in comparison to the effect of 100 μM nicotine administered in Ringer's solution (NIC100 μM, n=5). Repeated measures analysis showed that MEC and DβE, but not MLA pre-treatment significantly reduced the effect of 100 μM nicotine (P<0.05) on intraspinal ACh release.

an increased release of intraspinal ACh (Höglund *et al.* 2000). In addition, we unexpectedly found that the nicotinic antagonists, mecamylamine, and DβE also induced an increased intraspinal ACh release. These results suggest that nicotine and the antagonists mecamylamine and DβE may act on different mechanisms to cause an increased release of intraspinal ACh.

There is abundant evidence for the presence of nAChRs in the spinal cord, particularly in the superficial laminae of the dorsal horn where nociceptive Aδ and C fibres terminate (Gillberg *et al*. 1988; Roberts *et al.* 1995; Khan *et al.* 1996; Marubio *et al.* 1999) and in other parts of the nociceptive system (Adem *et al.* 1989; Iwamoto 1991; Bitner *et al.* 1998; Flores 1998; Ryan & Loiacono 2000). There is also good evidence that the substances we used interact with nAChRs (Khan *et al.* 1994 & 1997; Sullivan *et al.* 1994).

Although some yet undiscovered interactions, between the drugs we used and transmitter release regulatory mechanisms, might confound our interpretation, one possible explanation of the observed effects is that nicotine and the antagonists mecamylamine, DβE, and MLA act on different nAChRs. This suggestion is supported by recent findings. Damaj *et al.* (1998) suggested after comparing the effects of mecamylamine and DβE on nAChR agonist induced antinociception that α_4B_2, but not α-bungarotoxin sensitive nAChRs, are of importance for mediation of the antinociceptive effect of nicotine. Khan *et al.* (2001) also found evidence for differently localised spinal nAChRs of which some regulate transmitter release from adrenergic bulbospinal terminals. More closely related to the present results are the findings presented by Cordero-Erausquin & Changeux (2001) that nicotine and the antagonists, mecamylamine and DβE all increased the release of serotonin. Cordero-Erausquin & Changeux (2001) suggested the presence of three different nAChR subtypes that, directly or indirectly via regulation of the activity in inhibitory interneurones, modify intraspinal serotonin release.

Intraspinally administrated nicotine (1 μM–1 mM) induced a dose-dependent increase in ACh release. This indicates that the nAChR nicotine acts on was not maximally stimulated by the endogenous ACh release, despite the inhibition of acetylcholine esterase by neostigmine. Since it is unlikely that a cholinergic receptor would mediate an increased ACh release from cholinergic neurons in the central nervous system, these results indicate that this particular receptor (Rec1) is localised on non-cholinergic neurones that subsequently increase intraspinal ACh release. Since Cordero-Erausquin & Changeux (2001) showed that nicotine in this concentration range causes an increased release of serotonin it is possible that serotonin is one regulatory transmitter of ACh release.

The nicotinic antagonists mecamylamine and DβE presumably inhibit nAChRs. To produce an increased ACh release after inhibition, this particular nAChR subtype (Rec2) would function as an autoreceptor or reside on inhibitory interneurones that indirectly, tonically, modify ACh release. Since nicotine increased the release of ACh it is however unlikely that, as we also observed when muscarinic agonists were dialysed (Höglund *et al.* 2000), spinal ACh release is autoreceptor regulated. These antagonists appear also to interact with Rec1, since mecamylamine or DβE pre-treatment attenuated the effect of 100 μM nicotine on ACh release. It is obvious that nicotine also may interact with Rec2, but the affinity of nicotine for Rec2 may be less than for Rec1.

MLA microdialysis, contrary to DβE and mecamylamine, decreased ACh release below the basal level. This suggests that a third MLA sensitive nAChR subtype (Rec3) might be involved in the regulation of basal ACh release. However, the fact that pre-administration with MLA did not influence the effect of 100 μM nicotine on ACh release the present data do not allow us to speculate about a third nAChR subtype.

After administration of 10 mM nicotine, the ACh release was initially increased dramatically but later decreased to a very low level. A similar effect of nicotine has previously been observed in microdialysis in the hippocampus and frontal cortex in rats by (Tani *et al.* 1998) who suggested that this is an effect of desensitisation. The present data may explain the relatively short antinociceptive effect of spinally administered epibatidine (Khan *et al.* 1998), and the reduction in pain sensitivity observed in both animal and human subjects exposed to nicotine chronically (Milgrom Friedman *et al.* 1983; Mousa *et al.* 1988).

In conclusion, the present study shows that nicotine, mecamylamine and DβE, but not MLA, increase intraspinal ACh release. One plausible explanation for these effects is that different subtypes of nAChRs present in spinal cord tonically regulate spinal ACh release either directly or indirectly via inhibitory interneurones. Some of the spinal nAChRs may be desensitised at high agonist concentrations, an effect that needs to be considered while developing analgesic agents targeting these receptors.

Acknowledgements

The presented work was supported by Swedish Match North Europe AB (2001 08), the Magnus Bergvall and Åke Wiberg foundations, and the Swedish Institute.

References

Adem, A., A. Nordberg, S. S. Jossan, V. Sara & P. G. Gillberg: Quantitative autoradiography of nicotinic receptors in large cryosections of human brain hemispheres. *Neurosci. Lett.* 1989, **101**, 247–252.

Billard, W., H. Binch, G. Crosby & R. D. McQuade: Identification of the primary muscarinic autoreceptor subtype in rat striatum as m2 through a correlation of *in vivo* microdialysis and *in vitro* receptor binding data. *J. Pharmacol. Exp. Therap.* 1995, **273**, 273–279.

Bitner, R. S., A. L. Nikkel, P. Curzon, S. P. Arneric, A. W. Bannon & M. W. Decker: Role of the nucleus raphe magnus in antinociception produced by ABT-594: immediate early gene responses possibly linked to neuronal nicotinic acetylcholine receptors on serotonergic neurons. *J. Neurosci.* 1998, **18**, 5426–5432.

Bouaziz, H., C. Tong & J. C. Eisenach: Postoperative analgesia from

intrathecal neostigmine in sheep. *Anesth. Analg.* 1995, **80**, 1140–1144.

Bouaziz, H., C. Tong, Y. Yoon, D. D. Hood & J. C. Eisenach: Intravenous opioids stimulate norepinephrine and acetylcholine release in spinal cord dorsal horn. *Anesthesiology* 1996, **84**, 143–154.

Chen, S. R. & H. L. Pan: Spinal endogenous acetylcholine contributes to the analgesic effect of systemic morphine in rats. *Anesthesiology* 2001, **95**, 525–530.

Christensen, M. K. & D. F. Smith: Antinociceptive effects of the stereoisomers of nicotine given intrathecally in spinal rats. *J. Neural. Transm. Gen. Sect.* 1990, **80**, 189–194.

Cordero-Erausquin, M. & J. Changeux: Tonic nicotinic modulation of serotoninergic transmission in the spinal cord. *Proc. Natl. Acad. Sci. U S A*, 2001, **98**, 2803–2807.

Damaj, M. I., M. Fei-Yin, M. Dukat, R. A. Glennon & B. R. Martin: Antinociceptive responses to nicotinic acetylcholine receptor ligands after systemic and intrathecal administration in mice. *J. Pharmacol. Exp. Ther.ap* 1998, **284**, 1058–1065.

Damsma, G., B. H. Westerink, P. de Boer, J. B. de Vries & A. S. Horn: Basal acetylcholine release in freely moving rats detected by on-line trans-striatal dialysis: pharmacological aspects. *Life Sci.* 1988, **43**, 1161–1168.

Damsma, G., H. C. Westerink, J. B. de Vries, C. J. Van den Berg & A. S. Horn: Measurement of acetylcholine release in freely moving rats by means of automated intracerebral dialysis. *J. Neurochem.* 1987, **48**, 1523–1528.

Decker, M. W., P. Curzon, M. W. Holladay, A. L. Nikkel, R. S. Bitner, A. W. Bannon, D. L. Donnelly Roberts, P. S. Puttfarcken, T. A. Kuntzweiler, C. A. Briggs, M. Williams & S. P. Arneric: The role of neuronal nicotinic acetylcholine receptors in antinociception: effects of ABT-594. *J. Physiol. Paris* 1998, **92**, 221–224.

Flores, C. H. & K. M. Hargreaves: Neuronal nicotinic receptors: New targets in the treatment of pain. In: *Neuronal nicotinic receptors: Pharmacology and therapeutic opportunities.* Eds.: S. P. Arnerie & J. D. Brioni. Wiley-liss Inc., 1998, pp. 359–378.

Fürst, S.: Transmitters involved in antinociception in the spinal cord. *Brain. Res. Bull.* 1999, **48**, 129–141.

Gillberg, P. G., R. d'Argy & S. M. Aquilonius: Autoradiographic distribution of [3H]acetylcholine binding sites in the cervical spinal cord of man and some other species. *Neurosci. Lett.* 1988, **90**, 197–202.

Hama, A. T., G. K. Lloyd & F. Menzaghi: The antinociceptive effect of intrathecal administration of epibatidine with clonidine or neostigmine in the formalin test in rats. *Pain* 2001, **91**, 131–138.

Höglund, A. U., C. Hamilton & L. Lindblom: Effects of microdialyzed oxotremorine, carbachol, epibatidine, and scopolamine on intraspinal release of acetylcholine in the rat. *J. Pharmacol. Exp. Therap.* 2000, **295**, 100–104.

Iwamoto, E. T.: Characterization of the antinociception induced by nicotine in the pedunculopontine tegmental nucleus and the nucleus raphe magnus. *J. Pharmacol. Exp. Therap.* 1991, **257**, 120–133.

Jurna, I., P. Krauss & J. Baldauf: Depression by nicotine of pain-related nociceptive activity in the rat thalamus and spinal cord. *Clin. Investig.* 1993, **72**, 65–73.

Khan, I. M., H. Buerkle, P. Taylor & T. L. Yaksh: Nociceptive and antinociceptive responses to intrathecally administered nicotinic agonists. *Neuropharmacology* 1998, **37**, 1515–1525.

Khan, I. M., S. Stanislaus, L. Zhang, P. Taylor & T. L. Yaksh: A-85380 and epibatidine each interact with disparate spinal nicotinic receptor subtypes to achieve analgesia and nociception. *J. Pharmacol. Exp. Therap.* 2001, **297**, 230–239.

Khan, I. M., T. L. Yaksh & P. Taylor: Ligand specificity of nicotinic acetylcholine receptors in rat spinal cord: studies with nicotine and cytisine. *J. Pharmacol. Exp. Therap.* 1994, **270**, 159–166.

Khan, I. M., T. L. Yaksh & P. Taylor: Epibatidine binding sites and activity in the spinal cord. *Brain Res.* 1997, **753**, 269–282.

Khan, I. M., K. L. Youngblood, M. P. Printz, T. L. Yaksh & P. Taylor: Spinal nicotinic receptor expression in spontaneously hypertensive rats. *Hypertension* 1996, **28**, 1093–1099.

Klimscha, W., C. Tong & J. C. Eisenach: Intrathecal alpha 2–adrenergic agonists stimulate acetylcholine and norepinephrine release from the spinal cord dorsal horn in sheep. An in vivo microdialysis study. *Anesthesiology* 1997, **87**, 110–116.

Lawand, N. B., Y. Lu & K. N. Westlund: Nicotinic cholinergic receptors: potential targets for inflammatory pain relief. *Pain* 1999, **80**, 291–299.

Li, P. & M. Zhuo: Cholinergic, noradrenergic, and serotonergic inhibition of synaptic transmission in spinal lumbar dorsal horn of rat. *Brain Res. Bull.* 2001, **54**, 639–647.

Machelska, M., F. Pavone, F. Capone & B. Przewlocka: Antinocicepiton after both peripheral and intrathecal injection of oxotremorine is modulated by spinal nitric oxide. *Eur. Neuropsychopharm.* 1999, **9**, 213–217.

Marsala, M., A. B. Malmberg & T. L. Yaksh: The spinal loop dialysis catheter: characterizeation of use in the unanesthetized rat. *J. Neurosci. Meth.* 1995, **62**, 43–53.

Marubio, L. M., M. del Mar Arroyo Jimenez, M. Cordero Erausquin, C. Lena, N. Le Novere, A. de Kerchove d'Exaerde, M. Huchet, M. I. Damaj & J. P. Changeux: Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. *Nature* 1999, **398**, 805–810.

Meyer, M. D., M. W. Decker, L. E. Rueter, D. J. Anderson, M. J. Dart, K. H. Kim, J. P. Sullivan & M. Williams: The identification of novel structural compound classes exhibiting high affinity for neuronal nicotinic acetylcholine receptors and analgesic efficacy in preclinical models of pain. *Eur. J. Pharmacol.* 2000, **393**, 171–177.

Milgrom Friedman, J., R. Penman & R. Meares: A preliminary study on pain perception and tobacco smoking. *Clin. Exp. Pharmacol. Physiol.* 1983, **10**, 161–169.

Moor, E., P. DeBoer, F. Auth & B. H. C. Westerink: Characterisation of muscarinic autoreceptors in the septo-hippocampal system of the rat: a microdialysis study. *Eur. J. Pharmacol.* 1995, **294**, 155–161.

Mousa, S. A., V. J. Aloyo & G. R. Van Loon: Tolerance to tobacco smoke- and nicotine-induced analgesia in rats. *Pharmacol. Biochem. Behav.* 1988, **31**, 265–268.

Naguib, M. & T. L. Yaksh: Characterization of muscarinic receptor subtypes that mediate antinociception in the rat spinal cord. *Anesth. Analg.* 1997, **85**, 847–853.

Roberts, R. G., J. E. Stevenson, R. A. Westerman & J. Pennefather: Nicotinic acetylcholine receptors on capsaicin-sensitive nerves. *Neuroreport* 1995, **6**, 1578–1582.

Rogers, D. T. & E. T. Iwamoto: Multiple spinal mediators in parenteral nicotine-induced antinociception. *J. Pharmacol. Exp. Therap.* 1993, **267**, 341–349.

Roth, M. T., M. A. Fleegal, R. Lydic & H. A. Baghdoyan: Pontine acetylcholine release is regulated by muscarinic autoreceptors. *Neuroreport* 1996, **7**, 3069–3072.

Ryan, R. E. & R. E. Loiacono: Nicotinic receptor subunit mRNA in the thalamus of the rat: relevance to schizophrenia. *Neuroreport* 2000, **11**, 3693–3698.

Smith, M. D., X. H. Yang, J. Y. Nha & J. J. Buccafusco: Antinociceptive effect of spinal cholinergic stimulation: interaction with substance P. *Life Sci.* 1989, **45**, 1255–1261.

Sullivan, J. P., M. W. Decker, J. D. Brioni, D. Donnelly Roberts, D. J. Anderson, A. W. Bannon, C. H. Kang, P. Adams, M. Piattoni Kaplan, M. J. Buckley, et al.: (+/−)-Epibatidine elicits a diversity of *in vitro* and *in vivo* effects mediated by nicotinic acetylcholine receptors. *J. Pharmacol. Exp. Therap.* 1994, **271**, 624–631.

Tani, Y., K. Saito, M. Imoto & T. Ohno: Pharmacological characterization of nicotinic receptor-mediated acetylcholine release in rat brain – an *in vivo* microdialysis study. *Eur. J. Pharmacol.* 1998, **351**, 181–188.