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Compensation of muscarinic bronchial effects of talsaclidine by concomitant sympathetic activation in guinea pigs

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Abstract

The aim of the present investigation was to determine the reasons why the muscarinic receptor agonist talsaclidine (WAL 2014 FU, 1-azabicyclo[2.2.2] octane,3-(2-propynyloxy)-, (R)-,(E)-2-butenedioate) is devoid of bronchospastic effects in anaesthetized guinea pigs but causes contracture in isolated tracheal muscle from this species. Effects on airway resistance were assessed with a modified Konzett–Rössler method in guinea pigs anaesthetized with urethane. Intravenous injection of 1–64 mg/kg talsaclidine did not cause substantial bronchospasm in control animals. After blockade of β-adrenoceptors, the muscarinic receptor agonist induced dose-dependent bronchospasm which could be blocked by atropine. In despinalized animals and in animals with spinal transection, talsaclidine was bronchospastic but ED_{50} values were higher and maximal effects were smaller than in intact animals after β-adrenoceptor blockade. In adrenalectomized guinea pigs, talsaclidine was nearly as bronchospastic as after blockade of β-adrenoceptors. In contrast, the muscarinic ganglion stimulant McN-A-343, 4-(*m*-chlorophenylcarbamoyloxy)-2-butyn-trimethyl-ammonium chloride, (2–32 mg/kg i.v.), which has a muscarinic receptor profile similar to that of talsaclidine, i.e., full muscarinic agonism and highest affinity at muscarinic M_1 receptors, partial agonism at muscarinic M_3 receptors, but in contrast to talsaclidine does not penetrate the blood–brain barrier, caused dose-dependent bronchospasm in control animals. These results indicate that talsaclidine has bronchospastic potential which, however, does not become evident in vivo because of functional antagonism via β-adrenoceptors resulting from concomitant activation of the sympathetic nervous system in general and the adrenals in particular. It can be concluded that the unique profile of action of talsaclidine is due to partial agonism at bronchial muscarinic M_3 receptors, a prerequisite for susceptibility to functional antagonism, and to its ability to penetrate the blood–brain barrier readily and to induce sympathetic activation as a result of full agonism at peripheral ganglionic and adrenal as well as central muscarinic M_1 receptors. © 1997 Elsevier Science B.V.

Keywords: Talsaclidine; Arecoline; McN-A-343; Noradrenaline; Bronchospasm; Guinea pig

1. Introduction

Dementia in patients with Alzheimer's disease seems to be causally related with the loss of cholinergic neurones in the basal forebrain. Treatment with muscarinic receptor agonists has been attempted repeatedly. However, full clinical exploration of this therapeutic principle has been limited by side effects (Kumar and Calache, 1991). Talsaclidine (WAL 2014 FU) is a novel muscarinic receptor agonist and a new candidate for cholinergic replacement therapy. Its action profile in pharmacological experiments promises fewer and less severe side effects (Ensinger et al., 1993).

Talsaclidine has been characterized in isolated tissues as a full agonist at muscarinic M_1 receptors, but as a partial agonist at muscarinic M_2 and M_3 receptors. The compound is more potent at muscarinic M_1 than at M_2 and M_3 receptors (Ensinger et al., 1993, 1997).

In isolated tracheal muscle from guinea pigs, talsaclidine has contractile effects. The maximum contraction achievable amounted to 63% of that of carbachol; that of arecoline amounted to 114% (Walland and Palluk, 1994; Walland and Hammer, 1997). In anaesthetized guinea pigs, however, talsaclidine, in contrast to arecoline, even at high intravenous doses did not cause marked increases in airway resistance (Ensinger et al., 1993). We confirmed the lack of bronchospastic activity of intravenous talsaclidine in this species over the whole dose-range up to and beyond doses which arrest spontaneous respiration (Walland and Palluk, 1994).

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The present investigation was designed to elucidate the discrepancy between in vitro and in vivo results. Talsaclidine-induced contraction in isolated guinea-pig tracheal muscle is highly susceptible to antagonism by noradrenaline (Walland and Hammer, 1997). However, the muscarinic receptor agonist talsaclidine activates the sympathetic system by stimulation of ganglionic muscarinic M_1 receptors (Ensinger et al., 1993). We, therefore, investigated the importance of the sympathetic nervous system for the lack of bronchospastic effects in response to intravenous administration of talsaclidine in anaesthetized guinea pigs.

2. Materials and methods

2.1. Animals

Male and female guinea pigs, strain: Chbb.DHP, were obtained from the Experimental Animal Breeding Centre of Dr. Karl Thomae (Biberach, Germany). After fasting overnight, but with free access to drinking water, animals with a body weight of 400–500 g were used.

2.2. Preparation

Anaesthesia was induced by intraperitoneal injection of 1.5 g/kg urethane. Further doses of 0.15 g/kg were injected subcutaneously whenever the animals attempted to breath against the ventilator. A tracheal cannula was introduced after tracheotomy for artificial ventilation. The jugular vein was cannulated with a polyethylene tube for intravenous injection. Despinalization was performed by introduction of a metal rod from C_1 into the spinal canal and complete destruction of the spinal cord with an appropriate bottle brush provided with short but stiff bristles. Spinal transection was done at C_1 by blunt transection of the spinal cord with a metal hook. Bleeding and oozing of the wound was prevented by pressing a cone of plasticine into the spinal canal at C_1. Despinalized animals and animals with spinal transection were vagotomized by bilateral transection of the vagosympathetic trunks in the neck in order to exclude influences from autonomic centres completely. For transection of skin and muscular tissue a high-frequency cautery was used. Access to the spinal canal was gained by using a dental drill. Adrenalectomy was performed after bilateral laparotomy by ligation and removal of both adrenal glands.

2.3. Instrumentation and recording

Bronchospasm was recorded with a modified version of the method of Konzett and Rössler (1940). The animals were ventilated by means of a piston pump (7025 rodent ventilator, Ugo Basile, Varese, Italy) at a stroke volume of 10 ml/kg body weight and at a rate of 60 strokes per min. The tubing which connected the tracheal cannula with the ventilator was provided with a branch leading to a piston recorder (Fig. 1). The movement of the piston, i.e., the volume displacement, during inspiration was recorded with a lever system, a force transducer FT 03C (Grass, Quincy, MA, USA) and a Graphtec Multicorder MC 6625 (Graphtec, Tokyo, Japan). The piston recorder was housed in a pressurized perspex chamber, the air pressure being maintained at a constant level of 12–15 cm water by means of a membrane pump and a water valve (Fig. 1). This pressure

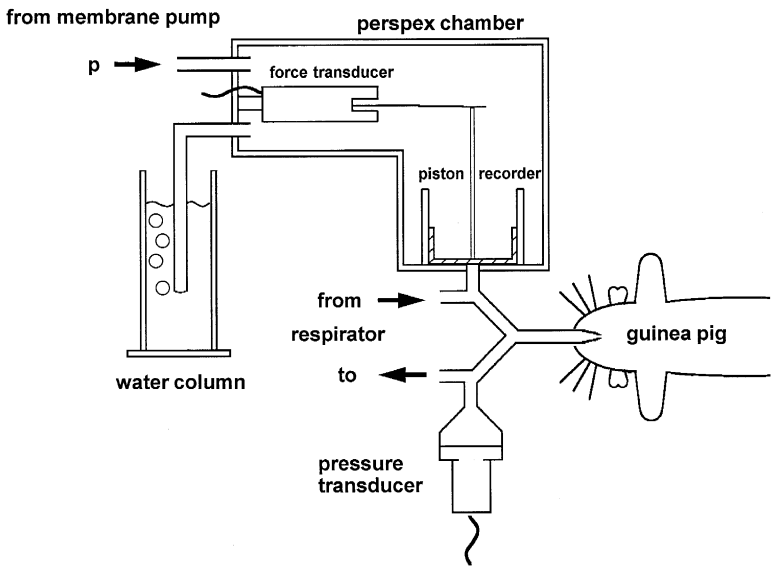

Fig. 1. Principle of the modified Konzett–Rössler method for measurement of bronchospasm. The pressure within the perspex chamber was elevated above atmospheric pressure by means of a membrane pump and was maintained at a constant level of 12–15 cm water by means of a water valve. This pressure was sufficient to balance the pressure in the breathing circuit so that a minimal overflow of air resulted under basal conditions (no bronchoconstriction). The lung and the piston recorder were deflated via the valve of the respirator (Starling pump).

was sufficient to balance the pressure in the breathing circuit so that a minimal overflow of air resulted under basal conditions (no bronchoconstriction). The lung and the piston recorder were deflated via the valve of the ventilator.

The original method of Konzett and Rössler (1940), which can be successfully used in larger animals at a lower ventilation rate, suffers in guinea pigs at higher ventilation rates from kinetic phenomena owing to the movement of water within the water valve. The present method is devoid of this movement because the permanent overflow of air does not allow intrusion of water into the pipe.

Blood pressure and heart rate were monitored from a carotid artery in order to check the viability of the preparation.

2.4. Experimental protocol

After allowing the preparation 20–30 min for stabilization after pharmacological pretreatment and 90 min after spinal transection, despinalization or adrenalectomy, muscarinic agonists were injected intravenously in increasing doses until maximum effects were obtained. Between injections there was a period of 10 to 45 min to allow effects to subside. Each group comprised 5–6 guinea pigs.

2.5. Evaluation

The maximum changes in respiratory overflow induced by the muscarinic agonist were evaluated, related to 100 g body weight and correlated with the logarithm of the dose. Fitting of the sigmoidal individual dose–response function was achieved by using the method of least squares and the equation (Bowman and Rand, 1980):

$$\text{effect} = B * (10^S)^{nH} / \left((10^C)^{nH} + (10^S)^{nH}\right)$$

where B = maximum effect, $C = \log \mathrm{ED}_{50}$ in mg/kg, nH = Hill-coefficient, $S = \log$ agonist dose in mg/kg.

Medians of the log ED_{50}, the maximum effects and the Hill-coefficients were calculated and used for calculation and graphical depiction of sigmoid curves together with the mean effects and standard errors for each dose. If no sigmoid curve could be fitted, the medians of the observations were plotted.

2.6. Statistics

Statistical comparisons of the effects of a compound after the different pretreatments were performed with the Kruskal–Wallis test. If this test resulted in a P-value less than 0.1, all interesting pairs were compared with a Wilcoxon-rank sum test (two-sided, normal approximation with continuity correction). The level of significance was set at 0.05.

All calculations were performed with the software product SAS (SAS Institute, Cary, NC, USA), version 6.04 on a PC HP-Vectra.

2.7. Chemicals and drugs

Urethane was purchased from Aldrich (Steinheim, Germany). Talsaclidine (WAL 2014 FU), 1-azabicyclo[2.2.2]octane,3-(2-propynyloxy)-, (R)-,(E)-2-butenedioate; arecoline, toliprolol (KOE 592, 1-(isopropylamino)-3-(*m*-tolyloxy-2-propanol-hydrochloride) and atropine were from Boehringer Ingelheim. McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butyn-trimethyl-ammonium chloride), DMPP (1,1-dimethyl-4-phenylpiperazinium) and hexamethonium were bought from Sigma (Deisenhofen, Germany).

3. Results

3.1. Talsaclidine in unpretreated guinea pigs

Intravenous injection of 1 to 64 mg/kg talsaclidine induced only very slight increases in respiratory overflow, indicating very faint if any bronchospastic activity of the drug (Fig. 2). After 1 mg/kg overflow even decreased in 5/6 animals.

3.2. Talsaclidine after blockade of β-adrenoceptors

Intravenous injection of 5 mg/kg toliprolol, a non-selective β-adrenolytic compound (Engelhardt and Traunecker, 1969), did not affect respiratory overflow in the anaesthetized guinea pig. After pretreatment with toliprolol, intravenous injection of talsaclidine resulted in full

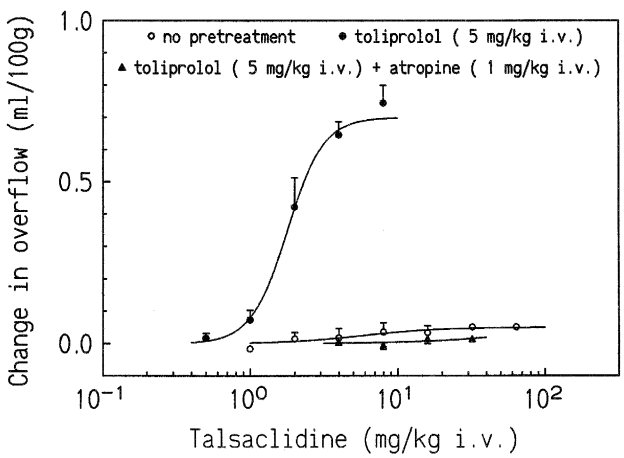

Fig. 2. Maximal changes in overflow in guinea-pig Konzett–Rössler preparations in response to intravenous injections of talsaclidine are presented. Results within groups of 5–6 animals each without pretreatment, β-blockade and combined β- and muscarinic blockade have been averaged (means ± SEM). Sigmoid curves have been fitted individually. The curves presented were calculated from medians.

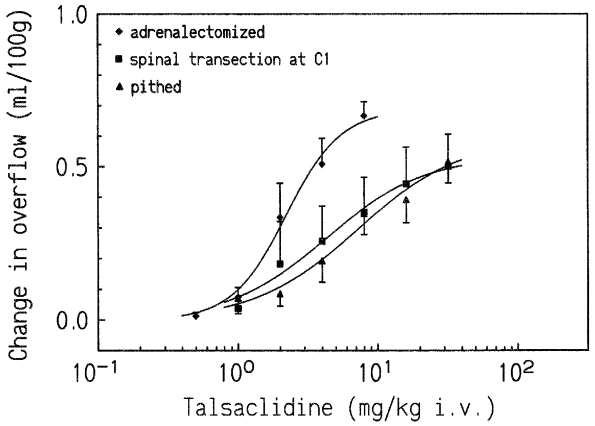

Fig. 3. Bronchospastic actions of talsaclidine in groups of 6 guinea pigs each with adrenalectomy, spinal transection at C_1 and despinalization (pithed). For technical details see Fig. 2 and methods.

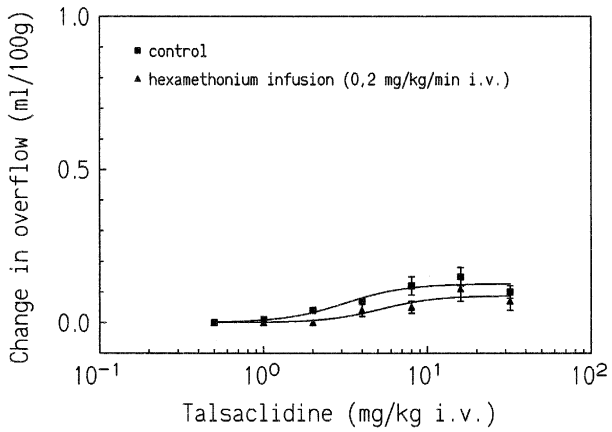

Fig. 4. Maximal changes in overflow in groups of Konzett–Rössler preparations from 6 guinea pigs, each in response to intravenous injection of talsaclidine without and during intravenous infusion of 0.2 mg/kg/min hexamethonium for blockade of nicotinic receptors. For technical details see Fig. 2 and Section 2.

bronchospasm which developed dose dependently (Fig. 2). A dose of 8 mg/kg talsaclidine induced within seconds maximal bronchospasm which was followed by hypotension and cardiac arrest.

3.3. Talsaclidine after blockade of muscarinic and β-adrenoceptors

After the blockade of β-adrenergic and muscarinic receptors with 5 mg/kg toliprolol i.v. and 1 mg/kg atropine i.v., talsaclidine at intravenous doses of 4–64 mg/kg did not induce bronchospasm at all (Fig. 2).

3.4. Talsaclidine in adrenalectomized guinea pigs

In guinea pigs with bilateral adrenalectomy, the intravenous injection of talsaclidine dose dependently induced bronchospasm (Fig. 3). The bronchospastic activity of talsaclidine was statistically indistinguishable from that seen in the experiments with blockade of β-adrenoceptors (Figs. 2 and 5).

3.5. Talsaclidine in despinalized guinea pigs

In despinalized guinea pigs talsaclidine dose dependently induced bronchospasm. However, the maximum effect appeared to be smaller than that in animals with β-adrenoceptor blockade, but the difference did not attain statistical significance. Higher doses were needed for half-maximal effects ($P < 0.03$) and Hill coefficients were smaller ($P < 0.03$) than in animals with β-adrenoceptor blockade (Figs. 2, 3 and 5).

3.6. Talsaclidine in guinea pigs with high level spinal transection

In guinea pigs with spinal cord transection at C_1, intravenous injections of talsaclidine induced dose dependent bronchospasm. The dose-response curve was quite similar to that for animals with despinalization (Fig. 3) and the differences did not attain statistical significance.

3.7. Talsaclidine after nicotinic receptor blockade

An intravenous infusion of 0.2 mg/kg/min hexamethonium influenced neither bronchotracheal resistance by itself nor the effect of talsaclidine (Fig. 4). At this infusion rate hexamethonium significantly decreased the pressor effect of 100 μg/kg i.v. of the nicotinic ganglion stimulant DMPP from 38 ± 10.1 to 9 ± 2.1 mm Hg, ($P < 0.05$, $N = 5$).

3.8. Arecoline in unpretreated guinea pigs

Intravenous injections of 0.01 to 0.16 mg/kg arecoline induced dose dependent bronchospasm. The highest dose caused maximum spasm and cardiac arrest (Fig. 6).

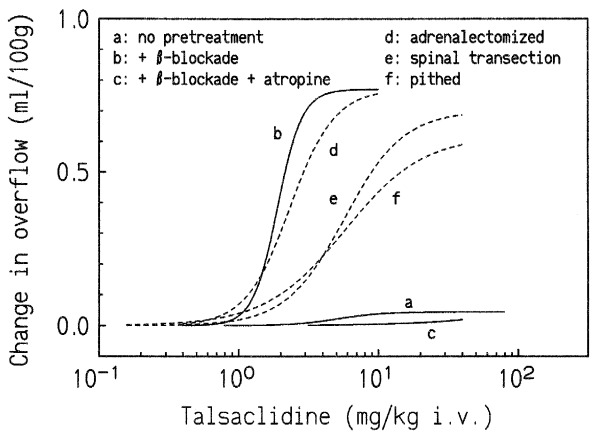

Fig. 5. Comparison of the influence of various pretreatments on the bronchospastic effect of talsaclidine in the guinea pig by superimposing the curves from Figs. 2 and 3.

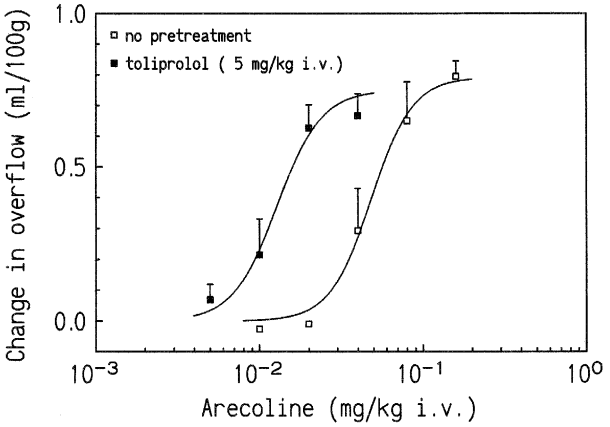

Fig. 6. Bronchospastic actions of the muscarinic agonist arecoline in groups of 6 guinea pigs each without and with β-blockade. For technical details see Fig. 2 and Section 2.

3.9. Arecoline after blockade of β-adrenoceptors

Intravenous pretreatment of guinea pigs with 5 mg/kg toliprolol, to produce non-selective blockade of β-adrenoceptors, decreased the bronchospastic ED_{50} of arecoline 3.7 fold ($P < 0.01$, Fig. 6).

3.10. McN-A-343 in unpretreated guinea pigs

Intravenous injection of 2–32 mg/kg McN-A-343, a muscarinic ganglion stimulant, caused bronchospasm in a dose dependent fashion (Fig. 7) and eventually cardiac arrest.

3.11. McN-A-343 after blockade of β-adrenoceptors

The pretreatment with 5 mg/kg toliprolol, to cause non-selective β-adrenoceptor blockade, potentiated the bronchospastic activity of McN-A-343, causing a 37.2-fold reduction ($P < 0.01$) of the ED_{50} (Fig. 7).

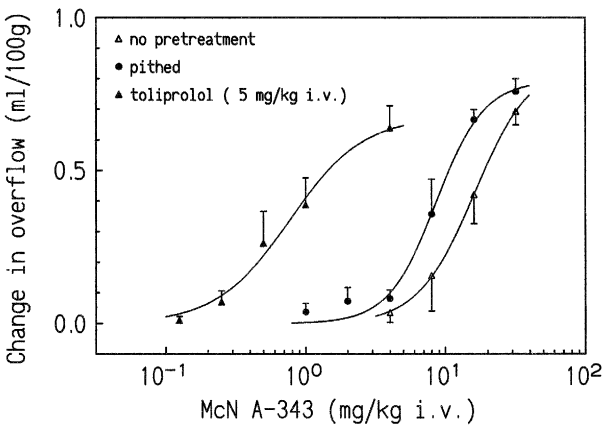

Fig. 7. Bronchospastic effects of the muscarinic ganglion stimulant McN-A-343 in 6 controls and 5 guinea pigs each with β-blockade and despinalization (pithed) respectively. For technical details see Fig. 2 and Section 2.

3.12. McN-A-343 in despinalized guinea pigs

Despinalization did not substantially increase the bronchospastic potency of McN-A-343. The reduction of the ED_{50} was only 1.7-fold (Fig. 6). This change did not attain statistical significance.

4. Discussion

The present experiments confirm the bronchospastic effect of arecoline and the lack of bronchospasm following administration of talsaclidine in the anaesthetized guinea pig as reported by Ensinger et al. (1993). Even doses of intravenous talsaclidine which arrest spontaneous respiration (Walland and Palluk, 1994) did not induce relevant increases in airway resistance in the artificially respirated Konzett–Rössler preparation.

β-Adrenoceptor blockade with toliprolol revealed the bronchospastic potential of talsaclidine, as was to be expected from the results obtained for this muscarinic partial receptor agonist in isolated tracheal muscle (Walland and Hammer, 1997). The complete abolition of this bronchospastic effect by atropine indicates that the action of talsaclidine at the bronchotracheal effector is exclusively muscarinic in nature. These results show that talsaclidine can exert muscarinic bronchoconstrictor effects which are, however, masked in unpretreated animals by a concomitant activation of a β-adrenoceptor-mediated, functionally antagonistic mechanism.

A direct agonistic effect of talsaclidine at β-adrenoceptors can be excluded because the drug has no such affinity in binding studies (Ensinger, personal communication). However, muscarinic receptor agonists stimulate sympathetic ganglia and the adrenals (see Trendelenburg, 1967) by activation of postganglionic muscarinic M_1 receptors (see Mitchelson, 1988; Mutschler et al., 1995). This has been confirmed in the guinea pig (Buckley and Burnstock, 1986; Maclagan et al., 1989; Roberts and Newbury, 1990). As talsaclidine was almost as bronchospastic in adrenalectomized guinea pigs as after β-adrenoceptor blockade, it may be concluded that the broncholytic contribution of the adrenals is much greater than that from sympathetic ganglia and their corresponding nerve endings in the tracheobronchial tree. This conclusion is in accordance with results from experiments with guinea pigs in which histamine-induced bronchospasm was antagonized by electrical stimulation of the spinal cord (Ainsworth et al., 1982). Inhibition has been observed upon stimulation at the level of the 3rd and 4th thoracic vertebrae, from where sympathetic innervation of the bronchi originates. Maximum inhibition, however, resulted from stimulation of adrenal efferents between T_9 and T_{10}. These results indicate that the liberation of adrenal catecholamines is the most effective mechanism of physiological sympathetic broncholysis in the guinea pig.

In despinalized guinea pigs talsaclidine caused bronchospasm which was smaller in maximum extent and appeared at higher doses than that observed in intact animals with β-adrenoceptor blockade. This difference is in accordance with sympathetic activation of the despinalized preparation by talsaclidine. It is surprising that talsaclidine was at all bronchospastic in despinalized animals because the structures at the level of sympathetic ganglia and the adrenals, which have muscarinic M_1 receptors, should be functionally intact and responsive to the drug. Preganglionic nerve activity should be very low after despinalization. However, if preganglionic nerve activity is a prerequisite for the development of the full broncholytic effect of talsaclidine, the muscarinic ganglion stimulant McN-A-343 (Roskowski, 1961) should not induce bronchospasm in intact guinea pigs because the compound has a similar muscarinic activity profile as talsaclidine, full muscarinic agonism and highest affinity at muscarinic M_1 receptors but partial agonism at muscarinic M_3 receptors (see Mitchelson, 1988).

In contrast to the quaternary charged molecule of McN-A-343, which should be unable to cross the blood–brain barrier, talsaclidine has free access to the brain (Ensinger et al., 1993). Therefore, maximal activation of the sympathetic nervous system by talsaclidine acting at both central and peripheral sites best explains the lack of bronchospastic effects in unpretreated animals. As the dose-dependence of talsaclidine-induced bronchospasm was very similar in despinalized and in guinea pigs with spinal transection at the first cerebral vertebra, the central site of drug action has to be expected rostral of C_1. The assumption that a brain site promotes sympathetic activation is in agreement with numerous investigations providing evidence for activation of the sympathetic nervous system by central muscarinic receptors (see Buccafusco, 1996). Direct evidence of central sympathetic activation by talsaclidine has been derived from the hypertensive and tachycardic reactions of anaesthetized guinea pigs in response to intracerebroventricular injection of small amounts of the drug (unpublished results).

Talsaclidine also interacts with the nicotinic receptors, albeit with less affinity than with muscarinic receptors, as can be concluded from binding studies (Bechtel, private communication) and results obtained with the phrenic nerve-diaphragm preparation (Palluk, unpublished results). However, the blockade of peripheral nicotinic receptors by infusion of hexamethonium did not render talsaclidine bronchospastic. This result certainly does not exclude effects of talsaclidine at central nicotinic receptors because hexamethonium is a charged molecule, but the result does argue against an important nicotinic contribution at peripheral receptors.

In conclusion the present experiments in guinea pigs show that talsaclidine is a muscarinic receptor agonist which, after elimination of sympathetic influence, is capable of inducing bronchospasm in vivo. In unpretreated animals the bronchospastic properties of talsaclidine are completely neutralized by concomitant activation of the sympathetic nervous system and the adrenals in particular, via peripheral postganglionic and presumptive central facilitatory muscarinic M_1 receptors.

This unique action profile seems to be due to the following properties of talsaclidine:

(1) Excellent susceptibility of the bronchospastic effect of talsaclidine to β-adrenoceptor-mediated functional antagonism as a result of partial agonism at muscarinic M_3 receptors (Walland and Hammer, 1997).

(2) Prevailing sympathetic activation via peripheral and presumptive central muscarinic M_1 receptors as a result of (a) selectivity of talsaclidine for the muscarinic M_1 receptor and development of full agonism at muscarinic M_1 receptors, (b) the ability of talsaclidine to penetrate the blood–brain barrier readily (Ensinger et al., 1993) for activation of presumptive facilitatory receptors, particularly those on cerebral neurons controlling adrenal catecholamine secretion.

The bronchospastic actions of arecoline can be explained by the resistance of its bronchospastic effect to β-adrenoceptor-mediated functional antagonism (see introduction) and those of McN-A-343 by the inability of the compound to cross the blood–brain barrier for central sympathetic activation. The differences in potentiation of the bronchospasm by β-blockade (Figs. 6 and 7) could be due to differences in the extent of sympathetic activation and particularly in susceptibility to functional antagonism.

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