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Influence of mecamylamine and atropine on tolerance development to nicotine hypothermia in rats

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Nicotine hypothermia is known as a central effect of nicotine waning after repeated administration due to the development of tolerance. In the present experiments using female rats, this tolerance was attenuated by the concomitant administration of mecamylamine. Atropine was without effect. It is concluded that tolerance to this effect of nicotine implies its contact with specific sites within the cns.

It has been reported recently by Ebenezer (1983a), that the acute effects of nicotine on the spontaneous activity of rats could be reversed by pretreatment with atropine. The author concluded that the depressant effects of nicotine might be due to a central cholinergic action. In a second experiment (Ebenezer 1983b) it was shown that daily doses of atropine (1·0 mg kg^{-1} s.c.) before treatment with nicotine (0·4 and 0·8 mg kg^{-1} s.c.) were able to reduce the development of tolerance to the depressant action of nicotine. I have chosen nicotine-induced hypothermia to study the effects of mecamylamine and atropine on tolerance development.

Mansner et al (1974) studied the changes in rectal temperature of mice following s.c. injections of nicotine and found that 2·5 mg kg^{-1} of the drug produced a marked fall in body temperature. This effect could be abolished by pretreatment with mecamylamine, but not by atropine nor hexamethonium. Mansner et al (1974) also administered 5 mg kg^{-1} nicotine subchronically three times a day for four days. In the pretreated mice a challenge dose of 2·5 mg kg^{-1} nicotine revealed the existence of a marked tolerance to its hypothermic effect. The present experiments were made to evaluate the influence of mecamylamine and atropine on the tolerance-inducing effect of nicotine. In addition, the ability of atropine to block acute nicotine hypothermia was compared with its antagonism of oxotremorine action according to Resul et al (1982).

Methods

Experiment 1. 32 female Sprague-Dawley rats (230–260 g) were housed individually in Macrolone cages (type 3) and had free access to food and water throughout the experiment. Room temperature was kept constant at 22 °C. The automatic light rhythm was adjusted to supply artificial illumination between 8 am and 8 pm. Animals were divided into four groups (eight rats per group) and were treated at the same time at 4 pm in the following way. Group CO: saline (0·9% NaCl) 1 ml kg^{-1} i.p., followed by oxotremorine 0·8 mg kg^{-1} s.c. 20 min later; Group AO: atropine 4 mg kg^{-1} i.p. before oxotremorine 0·8 mg kg^{-1} s.c.; Group AC: atropine 4 mg kg^{-1} i.p. before saline 1 ml kg^{-1} s.c.; Group CC: saline 1 ml kg^{-1} i.p. before saline 1 ml kg^{-1} s.c. Body temperature was determined immediately before the second injection (oxotremorine resp. control) (t_1) and 30 min after this injection (t_2). The difference $t_1 - t_2$ (i.e. the fall in body temperature in K) was registered for each animal. Measurements were carried out by inserting the probe of a thermocouple (Atlas Thermovit 4400) 3·5 cm into the rat rectum and reading the temperature value after 20 s.

Experiment 2. 40 female Sprague-Dawley rats were maintained in the same way as in experiment 1. Animals (150–170 g) were divided into four groups (ten rats per group) and were treated twice daily (11 am and 4 pm) for four successive days in the following way. Group CN: saline 1 ml kg^{-1} i.p., followed by nicotine 2·5 mg kg^{-1} s.c. 20 min later; Group MN: mecamylamine-HCl 2 mg kg^{-1} i.p. before nicotine 2·5 mg kg^{-1} s.c.; Group AN: atropine 4 mg kg^{-1} i.p. before nicotine 2·5 mg kg^{-1} s.c.; Group CC: saline 1 ml kg^{-1} i.p. before saline 1 ml kg^{-1} s.c. On day five after this four-day pretreatment all animals were treated with 2·5 mg kg^{-1} nicotine (s.c.) and body temperature fall was registered in the same way as in experiment 1.

To study the acute influence of mecamylamine and atropine on nicotine hypothermia, an additional temperature fall measurement was made after the first session of pretreatment.

Oxotremorine was dissolved in a 1% monopotassium phosphate solution, all other drugs were in distilled water giving an injection volume of 1 ml kg^{-1}. Doses of nicotine, atropine and oxotremorine are expressed as free bases ((−)-nicotine tartrate, atropine sulphate and oxotremorine-base were used), for mecamylamine, doses are as the hydrochloride.

Results and discussion

Data obtained after oxotremorine are illustrated in Fig. 1A and demonstrate a marked antagonistic activity of atropine (group AO) against this conventional muscarinic agonist.

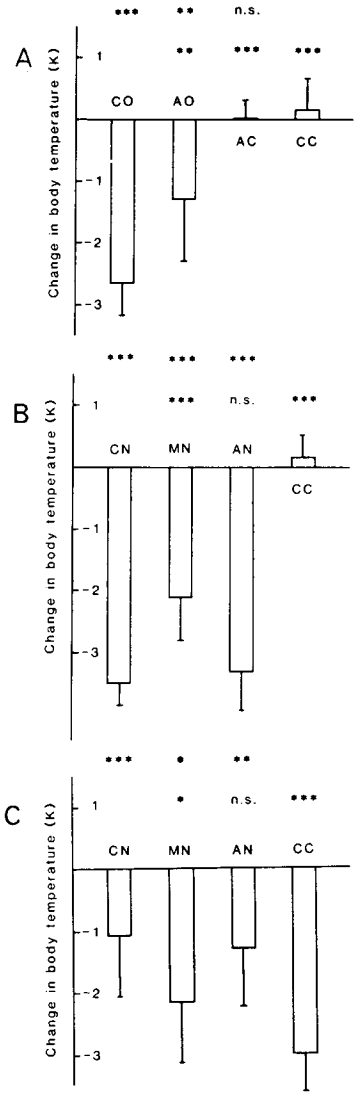

FIG. 1. A. Acute effect of treatment with saline 1 ml kg^{-1} i.p. followed by (20 min later) oxotremorine 0·8 mg kg^{-1} s.c. (CO), atropine 4 mg kg^{-1} i.p. followed by oxotremorine 0·8 mg kg^{-1} s.c. (AO), atropine 4 mg kg^{-1} i.p. followed by saline 1 ml kg^{-1} s.c. (AC) and saline 1 ml kg^{-1} i.p. before saline 1 ml kg^{-1} s.c. (CC) on rat body temperature, which was measured 30 min after the last injection. n = 8 rats. B. Fall in temperature after saline 1 ml kg^{-1} i.p. and 20 min later nicotine 2·5 mg kg^{-1} s.c. (CN), mecamylamine-HCl 2 mg kg^{-1} i.p. followed by nicotine 2·5 mg kg^{-1} s.c. (MN), atropine 4 mg kg^{-1} i.p. before nicotine 2·5 mg kg^{-1} s.c. (AN) and saline 1 ml kg^{-1} i.p. followed by saline 1 ml kg^{-1} s.c. (CC). Measurements as in A. n = 10 rats. C. Changes in temperature 30 min after a single nicotine challenge (2·5 mg kg^{-1} s.c.) on the day after a 4-day pretreatment (as under B twice daily for 4 days). Indicated are means ± s.d. The upper line of asterisks indicates significant differences to group CC, the lower line to group CO (in A) or group CN (B/C). *P <0·05, **P < 0·01 and ***P < 0·001. 'n.s.' means $P \geq 0{\cdot}05$ (Mann-Whitney-Wilcoxon-Test).

As shown in Fig. 1B, the same dose of atropine (group AN) was ineffective in blocking nicotine hypothermia. In contrast to this, the antagonistic efficacy of mecamylamine (group MN) was highly significant. After the four-day-pretreatment, all animals were given the same dose of nicotine alone. The resulting fall in body temperature is shown in Fig. 1C. Hypothermia was most evident in nicotine-naive animals (group CC). Whereas nicotine/saline-(CN) and nicotine/atropine-(AN) treated animals displayed an almost two-third-attenuation of response, mecamylamine (MN) markedly diminished tolerance development.

These results agree with those of other authors (Mansner et al 1974; Nordberg & Sundvall 1983), who found that mecamylamine effectively blocks nicotine-induced hypothermia. Atropine was used in a high pretreatment dosage (5 mg kg^{-1}) by Nordberg & Sundwall (1983) who considered it to be ineffective under their experimental conditions as we report here.

Since they found brain nicotine concentrations were affected neither by prior mecamylamine application nor by chronic pretreatment with high nicotine doses (5 mg kg^{-1} s.c., three times a day for four days), Mansner et al (1974) concluded that nicotine hypothermia is centrally mediated and that pharmacokinetic explanations for nicotine tolerance could not be derived from their results. This agrees with my earlier experiments (Horstmann 1982), in which rats under chronic treatment with nicotine displayed a marked increase in body temperature after an initial hypothermia within a few days. During this time, blood nicotine concentrations remained unchanged. The present results support the view that nicotine tolerance – particularly to its central actions – is of the true cellular type and can be blocked by preventing the drug acting intrinsically on nervous structures. Atropine doses of 4 mg kg^{-1}, which clearly antagonize fall in body temperature after oxotremorine, failed to alter both acute nicotine hypothermia and subsequent tolerance development.

The exact mechanism of nicotine tolerance remains unexplained. One of the possibilities is the involvement of brain catecholamines, as indicated by an increased noradrenaline turnover-rate after chronic nicotine treatment (Bhagat 1970).

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