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Nefopam but not physostigmine affects the thermoregulatory response in mice via α_2-adrenoceptors☆

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ABSTRACT

Nefopam, a non-opioid, centrally acting benzoxazocine analgesic, proved to be as efficient in treatment of postanaesthetic thermoregulatory shivering as clonidine or meperidine. However, its exact mechanism of action is still unclear. Potent anti-shivering activity was also demonstrated for physostigmine primarily based on cholinergic but probably also different additional mechanisms of action. Hypothesizing an involvement of α_2-adrenoceptors we studied their role in nefopam- and physostigmine-mediated thermoregulation in a mouse model of nonshivering thermogenesis. To differentiate possible α_2-adrenoceptor subtype-specific interactions, we analysed wildtype mice and mice with deletion of the α_{2A}-, α_{2B}- or α_{2C}-adrenoceptor (knock out).

Ten mice of each genotype ($n = 40$) were administered saline, saline plus atipamezole, 1 mg/kg nefopam, 25 mg/kg nefopam, 25 mg/kg nefopam plus atipamezole, physostigmine and physostigmine plus atipamezole intraperitoneally. Each mouse was randomly subjected to each of the seven different treatments. Afterwards, the mice were positioned into a plexiglas chamber where rectal temperature and mixed expired carbon dioxide were measured during following whole body cooling. Thermoregulatory threshold temperature of nonshivering thermogenesis and maximum response intensity were analysed.

Nefopam decreased the thermoregulatory threshold temperature in wildtype, α_{2B}- and α_{2C}-adrenoceptor mice. This effect was partially abolished by additional administration of the α_2-adrenoceptor antagonist atipamezole. In α_{2A}-adrenoceptor knock out mice, nefopam did not affect the thermoregulatory threshold. In contrast, physostigmine decreased the thermoregulatory threshold in wildtype and all α_2-adrenoceptor knock out mice independently from additional atipamezole administration.

Our results indicate an important role of the α_{2A}-adrenoceptor in the thermoregulatory response induced by nefopam but not by physostigmine in mice.

1. Introduction

Thermoregulatory shivering is a frequent problem in patients during the early postoperative period. Though the underlying mechanisms of shivering are not completely understood, a major contributing factor is perioperative hypothermia mediated by anaesthetic-induced inhibition of thermoregulation (Alfonsi, 2001; Sessler, 1997). Shivering is associated with increased metabolic rate which may result in dramatic increase of oxygen consumption (Macintyre et al., 1987) – a serious problem in patients with cardio- or cerebrovascular disease (Frank et al., 1995; Sessler, 1997).

Pharmacologically, shivering can be treated with different classes of drugs, including α_2-adrenoceptor agonists, certain opioids (e.g. meperidine) or others (e.g. nefopam or physostigmine) (Alfonsi et al., 1995; Horn et al., 1998; Piper et al., 1999). Nefopam is a non-opioid, non-steroidal centrally acting benzoxazocine analgesic that does not induce respiratory depression, considerable sedation or haemodynamic instability. Especially during the early postoperative period these are important properties accounting for the continuous interest in this drug (Evans et al., 2008; Fletcher et al., 2008; Troitzky et al., 2008). The mechanisms responsible for the thermoregulatory action of nefopam are not fully elucidated (Alfonsi et al., 2004). However, nefopam is able to block noradrenaline, 5-hydroxytriptamine and dopamine reuptake (Fuller and Snoddy, 1993; Rosland and Hole, 1990) and receptor

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binding studies revealed interaction with adrenergic α_1 and α_2-receptors, dopaminergic and serotoninergic receptors (Girard et al., 2006). In several clinical trials, nefopam was as effective or even superior for prevention and treatment of postanaesthetic shivering compared with the α_2-adrenoceptor agonist clonidine (Kranke et al., 2004; Piper et al., 2004, 1999).

The cholinesterase inhibitor physostigmine is commonly used to treat the central anticholinergic syndrome. It induces cholinergic stimulation of the hypothalamic-pituitary-adrenal axis and adrenal medulla and activates secretion of vasopressin, epinephrine and norepinephrine (Horn et al., 1998). In clinical studies, also physostigmine has been demonstrated to prevent postanaesthetic shivering as effective as meperidine, clonidine or nefopam (Horn et al., 1998; Rohm et al., 2005). However, the relationship between physostigmine and α_2-adrenergic pathways has not yet been elucidated with respect to thermoregulation.

Previous studies from our group have shown that meperidine's effect on thermoregulation in mice is partially mediated by α_2-adrenoceptors, especially the α_{2A}-subtype (Höcker et al., 2008a; Paris et al., 2005).

Therefore, the aim of our study was to analyse the role of α_2-adrenoceptors in nefopam- and physostigmine-mediated thermoregulation in mice. Our specific hypothesis was that the effect on thermoregulatory response exerted by nefopam and possibly also by physostigmine may be mediated – at least in part – via α_2-adrenoceptors. Because rodents try to resist hypothermia by nonshivering thermogenesis, we focused on a mouse model of nonshivering thermogenesis (Höcker et al., 2008a; Paris et al., 2005). To evaluate the individual role of the three α_2-adrenoceptor subtypes, we compared the impact of nefopam and physostigmine using wildtype mice and mice with deletion of the α_{2A}-, α_{2B}- or α_{2C}-adrenoceptor (knock out mice). To differentiate α_2-adrenoceptor-mediated effects from others we finally administered nefopam and physostigmine in combination with the α_2-adrenoceptor antagonist atipamezole.

2. Methods

2.1. Animals

Permission for this study was received from the local Institutional Review Board for animal research at the Ministry for Agriculture and Environment, Kiel, Germany, before the initiation of work. All experiments were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986, and the European Communities Council Directive of 24. November 1986. Further, all efforts were made to minimise animal suffering. The generation of mouse lines lacking a single α_2-adrenoceptor (α_{2A}-, α_{2B}- or α_{2C}-adrenoceptor knock out) has been described previously (Altman et al., 1999; Link et al., 1996). The different knock out mice were generated on different genetic backgrounds (α_{2A}-knock out: FVB/N, α_{2B}-knock out: 129Sv/J and C57BL6/J, α_{2C}-knock out: 129Sv/J and FVB/N). The knock out mice were obtained from L. Hein, Freiburg, Germany, and maintained in a specified pathogen-free facility at our institution where all experiments were performed. Wildtype mice (129S2/SvHsd) were analysed as a control group.

2.2. Experimental procedure

Ten mice of each genotype weighing 29–37 g were investigated. Mice were housed 3–4 animals per cage and maintained on a 12 h light–dark cycle with free access to water and food. All experiments were performed between 09:00 h and 16:00 h. The animals were weighed before the experiments. In a first set of experiments, animals received saline, nefopam (Acupan™, Biocodex, Gentilly Cedex, France) (1 mg/kg and 25 mg/kg) or physostigmine (3 mg/kg) (Anticholium™, Köhler-Chemie, Alsbach-Hähnlein, Germany), respectively. To evaluate if possible effects of nefopam or physostigmine are mediated via α_2-adrenoceptors, the α_2-adrenoceptor antagonist atipamezole (2 mg/kg) was injected 20 min after the administration of saline, nefopam or physostigmine. All drugs were administered intraperitoneally (0.1 ml/10 g body weight).

Each animal was analysed with all seven different drug regimens: [1] 'saline', [2] 'saline plus atipamezole', [3] 'nefopam low dose' (1 mg/kg), [4] 'nefopam high dose' (25 mg/kg), [5] 'nefopam high dose plus atipamezole', [6] 'physostigmine' and [7] 'physostigmine plus atipamezole' and therefore served as its own control. Before starting a new experiment, animals had at least 10 days to recover from injections of pharmaceuticals. After injection of saline, nefopam or physostigmine, respectively ('one drug trial'), the animals were returned to their cages until starting the measurements 40 min later. In a second set of experiments, 20 min after injection of saline (regimen [2] 'saline plus atipamezole'), nefopam (regimen [5] 'nefopam high dose plus atipamezole') or physostigmine (regimen [7] 'physostigmine plus atipamezole') as the first drug, mice were injected atipamezole as the second drug and immediately returned to their cages until start of measurements 20 min later ('two drug trial') (Fig. 1). To prevent an inappropriate decline of body temperature, cages were warmed by a heating lamp.

Forty minutes after injection of the first drug, respectively, mice were positioned in a gas-tight plexiglas chamber (internal diameter: 4 cm; length: 7 cm). A constant oxygen flow of 100 ml/min was delivered to the chamber. Mixed expired carbon dioxide was recorded continuously by capnography (Phasein Multigas sensor™, Armeda Medizintechnik, Hannover, Germany). Body temperature was measured and recorded continuously by a rectal temperature probe (Exacon-Asmuth, Minden, Germany; Sirecust 402™, Siemens, Danvers, MA). Ice was positioned around the plexiglas chamber to provoke a thermoregulatory response by whole body cooling (rate of temperature decrease 0.1–0.2 °C/min). No animal was allowed to get colder than 32 °C. According to previous studies in rats (Maurer et al., 2000) the thermoregulatory threshold was defined as the temperature (°C) at which a sustained increase in expiratory carbon dioxide can be observed (Fig. 1). The maximum response intensity of nonshivering thermogenesis was calculated as the ratio of the maximum and minimum carbon dioxide plateau during cooling.

2.3. Statistical and data analyses

To determine the thermoregulatory threshold, plots of mixed expired carbon dioxide (mmHg) as a function of time were analysed by three blinded observers and the median value of the three observers was taken for further calculations. Within-group data were analysed using repeated-measures analysis of variance (ANOVA), with Dunnett's test for comparison to control (precooling baseline values). Differences between the treatment groups were evaluated using one-way ANOVA followed by Bonferroni correction for multiple comparisons. To detect possible differential effects between different genotypes, a 4×7 ANOVA was performed. Results are presented as mean ± SD; $P < 0.05$ was considered significant. GraphPad Prism 5.0™ (GraphPad Software, San Diego, CA) was used to perform statistical analyses.

3. Results

Baseline body temperatures before and after intraperitoneal injections were comparable between mice of all groups and between wildtype and α_2-adrenoceptor knock out mice.

The thermoregulatory threshold temperature in wildtype mice receiving saline ('saline') was 36.0 ± 0.5 °C. Additional injection of atipamezole ('saline plus atipamezole') did not change threshold temperature significantly (36.0 ± 0.6 °C). After administration of 1 mg/kg nefopam ('low dose'), the thermoregulatory threshold decreased to 35.7 ± 0.7 °C. A dose of 25 mg/kg nefopam ('high dose') resulted in a significant decline of threshold temperature (34.8 ± 0.7 °C) ($P = 0.0009$ vs. saline). This effect could be abolished by additional administration of atipamezole ('nefopam high dose plus atipamezole') (35.4 ± 0.6 °C) (Fig. 2A) ($P = 0.027$ vs. 'nefopam high dose').

In α_{2B}- and α_{2C}-adrenoceptor knock out mice the thermoregulatory threshold temperature after injection of saline was comparable (36.3 ± 0.7 °C vs. 36.2 ± 0.6 °C). No significant changes could be detected in both groups after additional injection of atipamezole. Administration of 1 mg/kg nefopam did not induce any significant effect on threshold temperature in both α_{2B}- and α_{2C}-adrenoceptor knock out mice, whereas after administration of 25 mg/kg nefopam the threshold temperature decreased to 35.2 ± 0.7 °C (α_{2B}-adrenoceptor knock out; $P = 0.0002$ vs. saline) and 35.5 ± 0.5 °C (α_{2C}-adrenoceptor knock out; $P = 0.002$), respectively. Additional administration of atipamezole ('nefopam high dose plus atipamezole') abolished this effect in both groups of knock out mice (35.8 ± 0.7 °C) in α_{2B}- vs. 35.8 ± 0.6 °C in α_{2C}-adrenoceptor knock out mice (Fig. 2C,D).

In contrast, in α_{2A}-adrenoceptor knock out mice nefopam application in either dose did not result in a significant decline of the thermoregulatory threshold temperature (35.9 ± 0.7 °C versus 36.4 ± 0.6 °C in the saline control group). No significant