Interactions of intracerebroventricular pertussis toxin treatment with the ataxic and hypothermic effects of ethanol

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Summary. Pretreatment with pertussis toxin (0.5 and 1.0 μg/animal, i.c.v., seven days prior to testing) reversed the reduction in locomotor activity in the holeboard test caused by administration of the alpha2-adrenoceptor agonist, medetomidine (0.1 mg/kg, i.p.). Intrinsic behavioral effects of pertussis toxin treatment were also observed, these included a reduction in exploratory head-dipping and an increase in locomotor activity. These doses of pertussis toxin also reduced the ataxia induced by a 2.4 g/kg dose of ethanol. Pertussis toxin treated animals also exhibited a diminished hypothermic response to ethanol (2 g/kg), although the pertussis toxin treated animals had lower body temperatures prior to ethanol administration compared to sham treated animals. Neither the behavioral effect of pertussis holotoxin in the holeboard nor its effects on reversing medetomidine hypolocomotion or ethanol-induced ataxia were seen following administration of the binding (B) oligomer of pertussis toxin, which binds to, but does not penetrate, cell membranes. These findings implicate mechanisms involving pertussis toxin sensitive G-proteins in modulating some behavioral and physiological effects of ethanol.

Key words: Pertussis toxin — Pertussis toxin B-oligomer — G-proteins — Ethanol — Ataxia — Hypothermia

Introduction

Many neurotransmitter receptors are linked to their effector mechanisms via guanine nucleotide-binding proteins (G-proteins) (Gilman 1987; Worley et al. 1987). This linkage can be via second messenger systems such as production of cyclic adenosine monophosphate (cAMP) or inositol 1,4,5-trisphosphate (IP3) and diacylglycerol, or may involve actions on ions channels (Schramm and Selinger 1984; Cockcroft and Gomperts 1985; Holz et al. 1986). G-proteins can be inhibitory (Gi) or stimulatory (Gs), although other G-proteins (Gp) whose exact role is not fully understood also exist. Pertussis toxin inactivates Gi and Gs proteins by ADP-ribosylation and therefore uncouple receptors from their effector mechanisms (Uy et al. 1988).

Receptors linked to pertussis toxin-sensitive G-proteins include alpha2 adrenoceptors (Bloom 1978), adenosine A1 (Wojcik et al. 1985), 5-HT1A (Harrington et al. 1988), muscarinic M2 (Olianas et al. 1983), dopamine D2 (Cooper et al. 1986), GABA_b (Wojcik and Neff 1983), and, mu (Cooper et al. 1986) and sigma (Schoffelmeer et al. 1986) opiate receptors. Some of these receptors, such as alpha2 adrenoceptors (Durcan et al. 1989a, b, c, d; Lister et al. 1989), adenosine A1 receptors (Clark and Dar 1988; Dar et al. 1987) and GABA_b receptors (Allan and Harris 1989), have been implicated in the mediation of the behavioral and physiological effects of ethanol.

The aim of the present study was to assess the effect of pretreatment with pertussis toxin on the ataxic and hypothermic effects of ethanol. Additionally, the effects of the binding (B) oligomer of pertussis toxin, which binds to, but does not penetrate, cell membranes were also investigated in order to assess if this oligomer had actions similar to the enzymatically active holotoxin. The effect of pertussis toxin treatment on the motility reducing properties of medetomidine, which are thought to be mediated via Gi linked alpha2-adrenoceptors, was used to validate and probe the effectiveness of the pertussis toxin pretreatments. The interaction of pertussis toxin treatment with the ataxic and hypothermic effects of ethanol was investigated using a toxin regimen derived from the medetomidine studies. Ethanol was administered as in previous investigations (Durcan et al. 1989b, c, d; Lister et al. 1989).

Methods

Animals. Naive NIH Swiss male mice were housed in groups of 10 on a 12:12 hour light:dark cycle with food and water available ad
lib. The mice weighed between 25 and 28 g at the time of pertussis toxin or sham treatment. All mice were housed individually after pertussis toxin or sham treatment.

**Pertussis toxin treatment.** Mice were treated with pertussis holotoxin or pertussis toxin binding oligomer (List Biological Labs., Campbell, CA) injected in a volume of 5 μl directly into the lateral ventricle of the brain. Animals were anesthetized using chloral hydrate, a small incision made in the scalp, and the lateral ventricle accessed (co-ordinates from Bregma: 1 mm lateral, 1 mm rostral, -3 mm vertical). Pertussis toxin was injected at a rate of 5 μl/min for 1 min, the injection needle remained in place for a further 2 min before being slowly retracted and the wound closed using a wound clip. Sham animals were injected with the vehicle buffer in which the toxin was dissolved (0.01 M sodium phosphate, 0.05 M sodium chloride, pH 7.0).

**Holeboard testing.** The holeboard consisted of a Plexiglas box (40 x 40 x 30 cm), the floor of which had four equally spaced holes, 3 cm in diameter. In two opposite walls, 2 cm above the floor, were four equally spaced infra-red photoemitters to measure movement in the box. There were also photoemitters beneath each hole to measure the number and duration of head-dips. The apparatus was interfaced with a PDP-11 computer running SKED-11 software (State Systems Inc., Kalamazoo, MI).

The holeboard testing, which took place in a dimly lit room, involved placing a mouse in the center of the holeboard floor and tracking its movements using the photoemitters for 8 min.

**Ataxia testing.** Mice were injected i.p. with 2.4 g/kg ethanol (at a volume of 20 ml/kg) and after 5 min rated for ataxia using the following scale, modified from Majchrowicz (1975): 0 = no observable effect; 1 = mild ataxia; 2 = moderate ataxia; 3 = severe ataxia; 4 = very severe ataxia, only just able to recover righting reflex; 5 = loss of righting reflex.

**Temperature recording.** Core body temperatures were measured using a rectal probe and a digital thermometer (Sensortek Inc.). The probe was inserted 2.5 cm into the colon of each mouse.

**Interactions with medetomidine-induced hypomotility.** Groups of animals (N = 14–22) were treated with either pertussis toxin (1.0 μg/animal, i.c.v.) or with buffer. Two, four or seven days following pertussis toxin or sham treatment the animals were divided into approximately equal groups and injected with either 0.1 mg/kg medetomidine or distilled water vehicle 30 min prior to testing in the holeboard. This dose of medetomidine was chosen on the basis of previous studies (Durcan et al. 1989b) demonstrating a marked reduction in locomotor activity in the holeboard test. Separate sets of animals were used for each time point.

In a subsequent experiment groups of animals (N = 18–22) were injected i.c.v. with either pertussis holotoxin (1.0 μg/animal), pertussis toxin B-oligomer (1.0 μg/animal) or with buffer. Seven days following pertussis holotoxin, B-oligomer or sham treatment the animals were divided into approximately injected with either 0.1 mg/kg medetomidine or distilled water vehicle 30 min prior to testing in the holeboard.

In a different set of experiments, groups of animals were injected i.c.v. with two lower doses of pertussis toxin 7 days prior to testing in the holeboard. Mice (N = 18–20) were treated with 0.50 μg/animal pertussis toxin or with buffer. Seven days later half of each group were treated with either 0.1 mg/kg medetomidine (i.p.) or distilled water vehicle 30 min prior to testing in the holeboard. Separate groups of animals (N = 18–22) were treated with 0.25 μg/animal pertussis toxin or sham injected with buffer. Seven days later half of each group received either 0.1 mg/kg medetomidine (i.p.) or distilled water vehicle 30 min prior to testing in the holeboard.

**Interactions with ethanol-induced ataxia.** Groups of animals (N = 8–20) were treated with either 0.125, 0.250, 0.500 or 1.00 μg/animal or sham injected as described above. Seven days later all animals were injected i.p. with 2.4 g/kg ethanol dissolved in a distilled water vehicle. Every 5 min up to 25 min after the ethanol injection the animals were "blindly" rated for ataxia using the scale described above.

In a subsequent experiment, groups of animals (N = 10) were injected i.c.v. with either pertussis holotoxin (1.0 μg/animal), pertussis toxin B-oligomer (1.0 μg/animal) or with buffer. Seven days following pertussis holotoxin, B-oligomer or sham treatment all animals were injected i.p. with 2.4 g/kg ethanol dissolved in a distilled water vehicle. Every 5 min up to 25 min after the ethanol injection the animals were "blindly" rated for ataxia using the scale described above.

**Interactions with ethanol-induced hypothermia.** Groups of animals (N = 15) were pretreated with either 1.0 μg/animal pertussis toxin or sham treated as described above. Seven days later all animals were administered 2 g/kg ethanol (i.p.) immediately following a core body temperature recording. Twenty minutes later the body temperature of each animals was again measured.

**Blood ethanol determinations.** Groups of animals (N = 15) were pretreated with either 1.0 μg/animal pertussis toxin or sham treatment as described above. Seven days later these animals were administered ethanol (2 g/kg, i.p.) 20 min prior to decapitation. Trunk blood was collected in heparinized tubes. Blood ethanol concentrations were determined using a modification of the Sigma diagnostics alcohol procedure No. 332-UV (Sigma Chemical Co., St Louis, MO) which involves the conversion of ethanol to acetaldehyde and nicotinamide adenine dinucleotide (NAD) to NADH catalyzed by the enzyme alcohol dehydrogenase (Bucher and Redetzki 1951). The levels of NADH were measured photometrically.

**Statistics.** Data were analyzed using analysis of variance (with repeated measures when appropriate). When significant differences between groups were found from the ANOVA, post hoc comparisons between individual means were made using Fisher's least significant difference test.

**Results**

**Interactions with medetomidine-induced hypomotility.**

In all experiments medetomidine (0.1 mg/kg) significantly reduced both locomotor activity and exploratory head-dipping in sham treated animals.

Two days after i.c.v. administration of pertussis toxin no effect on locomotor activity was seen as compared to vehicle treated sham controls, although a significant (p < 0.05) reduction in head-dipping was detected (Fig. 1A). Four days following pertussis toxin or sham treatment, vehicle treated pertussis toxin animals showed significantly (p < 0.05) elevated locomotor activity and significantly (p < 0.01) reduced exploratory head-dipping compared to vehicle treated sham controls (Fig. 1B). However, the pertussis toxin pretreated animals were significantly (p < 0.05) less affected by the medetomidine than were the sham operated controls. Seven days after pertussis toxin or sham treatment medetomidine significantly (p < 0.001) reduced exploratory head-dipping in both groups but only reduced locomotor activity in the sham operated animals (Fig. 1C). Pertussis toxin treatment significantly (p < 0.05) elevated locomotor activity and significantly (p < 0.001) reduced exploratory as compared to sham-vehicle controls. However, a highly signifi-