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Central nicotinic receptor blockade inhibits emotionally conditioned pressor responses in rats

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Abstract. A conditioned stimulus previously paired with electric footshock produced an increase in blood pressure in conscious, freely moving rats. The conditioned pressor response was reproducible. Intracerebroventricular injection of the nicotinic receptor antagonists hexamethonium (1–10 μg) or pentolinium (10 μg) but not the muscarinic receptor antagonist methylatropine (3 μg) produced an inhibition of the conditioned pressor response, whereas intraarterial injection of hexamethonium (10 μg) did not affect the response. Intraventricular injection of the cholinesterase inhibitor physostigmine (3–10 μg) produced an enhancement of the conditioned pressor response. These results are consistent with the possibility that central nicotinic receptors play a role in the expression of the emotionally conditioned pressor response in rats.
Key words. Central nicotinic receptors; central muscarinic receptors; classical conditioning; conditioned pressor response; central acetylcholine.

Psychological stress has been shown to cause stimulation of the cardiovascular system and subsequent hypertension[1,2]. Although little is known of the exact central nervous system mechanisms generating the cardiovascular responses elicited by stress, acetylcholine in the brain is one of the neurotransmitters clearly involved in the physiological responses. Chronic stress causes an increase in the release of acetylcholine[3,4] and in the activity of choline acetyltransferase[5] in the brain.

Activation of central nicotinic receptors is similar to stress in various ways. For example, both stress and activation of central nicotinic receptors cause an increase in the release of corticotropin-releasing factor which is implicated in playing a major role in the expression of stress-induced behavioural and cardiovascular responses[6–8]. Both stress and central nicotinic receptor activation also increase the release of central catecholamines which are thought to be involved in mediation of physiological responses elicited by both stimuli[9–12]. In addition, chronic immobilization stress causes downregulation of central nicotinic receptors[13], suggesting that chronic stress results in an activation of central nicotinic receptors. On the other hand, chronic stress is reported to increase the number of central muscarinic receptors[3,14,15]. Both nicotinic and muscarinic agents acting centrally cause an increase in blood pressure[16–18].

These lines of evidence are compatible with the hypothesis that central nicotinic and/or muscarinic receptors are involved in mediation of physiological responses elicited by stress. In the present study in rats, we established a method for evaluating the effects of drugs on the pressor response elicited by a conditioned stimulus previously paired with footshock, a kind of psychological stress, and investigated whether central nicotinic and muscarinic receptors are involved in the expression of the emotionally conditioned pressor response.

Materials and methods

Male Wistar rats (290–330 g) were individually housed in plastic cages. They were kept under 12 h periods of dark and light, and given standard rat chow and tap water ad libitum. For experiments in waking rats, cannulae were implanted into the lateral brain ventricle and the abdominal aorta. The rats were then allowed to recover from the surgery for more than three days before each rat was used in subsequent experiments.

Implantation of chronic catheterization for cardiovascular recording. Rats were anesthetized with pentobarbital (50 mg/kg, i.p.). For recording arterial pressure, a polyethylene cannula (Natsume, Tokyo, Japan) (0.5 mm, i.d., connected to the polyethylene tubing of 0.85 mm, i.d.) filled with saline containing heparin (50 units/ml of 0.9% saline), was inserted into the abdominal aorta via the left femoral artery[19]. The other end was passed subcutaneously to emerge at the back of the neck, and the catheter was held in place with wound clips. During experiments, arterial pressure was recorded continuously by connecting the catheter to a pressure transducer.

Intracerebroventricular surgery. Intracerebroventricular injections were performed according to the method described by Buccafusco and Yang[20]. Rats were anesthetized with pentobarbital while a 10 mm guide cannula (23-gauge stainless steel tubing) was inserted into the left lateral brain ventricle. Through a burr hole, the cannula guide was placed 1.5 mm below the cortical

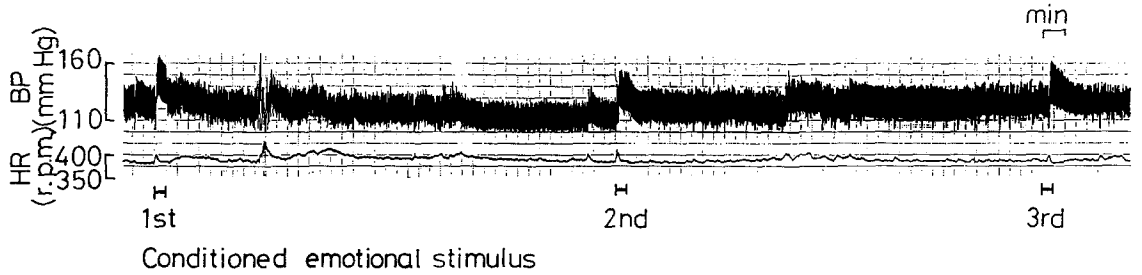

Figure 1. Tracing of arterial blood pressure (BP, mm Hg) and heart rate (HR, r.p.m.) from a rat, showing pressor responses elicited by the conditioned emotional stimulus. The conditioned stimulus was applied three times at intervals of 20 min.

surface so that the tip was just above the surface of the left lateral ventricle (0.8 mm caudal and 1.4 mm lateral to bregma). A steel screw with a covering of acrylic cement was used to anchor the guide in place, and a 30-gauge wire was used to plug the guide.

Intraventricular injection. In freely moving rats, a 30-gauge stainless steel injection cannula was connected to a 50 µl Hamilton syringe using polyethylene tubing. The cannula was lowered into the guide and drug solutions were injected. Each injection was a volume of 10 µl delivered manually in 15 s. Each rat had only one dose of one drug. After each experiment, methylene blue was injected through the injection cannula to verify correct placement of the cannula tip within the lateral ventricle.

Classical fear conditioning. Classical fear conditionings were performed by a modification of the method described by LeDoux et al.[21]. In brief, the rat was removed from its home cage and placed in a standard conditioning chamber. After a 5 min period of acclimatization, the conditioned emotional stimulus (800 Hz, 82 dB, 10 s pure auditory tone) was presented 40 times through a speaker mounted in the test chamber. The average intertrial interval was 150 s with a range between 100 and 198 s. During the first 10 trials, the tone was presented alone in order to habituate orienting responses and, over the following 30 trials, the termination of the tone was coextensive with a 2.0-second delivery of the electric footshock unconditioned stimulus (3 mA) distributed across the grid floor of the chamber.

Cardiovascular responses elicited by the conditioned fear stimulus were assessed during extinction trials (30 s tone conditioned emotional stimulus delivered without the footshock unconditioned stimulus). The conditioned emotional stimulus was presented three times every 20 min, beginning 15 min after the end of 40 trials. All drugs were administered 15 min before the second stimulus.

Serum levels of noradrenaline and adrenaline were measured radioenzymatically using catecholamine [^{3}H] research assay system (Amersham).

Drugs. (−)Nicotine, the nicotinic receptor antagonists pentolinium ditartrate, carbamyl-choline chloride (Sigma) and hexamethonium bromide, the cholinesterase inhibitor physostigmine sulfate (Tokyo Kasei, Tokyo, Japan) and the muscarinic receptor antagonist atropine methyl bromide (Takeda Pharmaceutical Co., Osaka, Japan) were dissolved in 0.9% saline. All dosages are expressed as the salt.

Data are presented as the mean ± SEM. Statistical studies, using analysis of variance and Student's t-test for individual differences, were performed.

Results

Resting mean arterial pressure and heart rate were 108 ± 2 mm Hg and 381 ± 8 beats/min, respectively, in 59 conscious freely moving rats. A conditioned stimulus previously paired with footshock consistently produced an increase in blood pressure (fig. 1). The pressor peak occurred within 10 s and the blood pressure remained elevated above baseline at 30–60 s following the stimulus onset. The pressor response was reproducible when the conditioned stimulus was evoked three times every 20 min (fig. 1). On the other hand, heart rate was slightly increased or unchanged following the stimulation.

Following the conditioned fear stimulus, plasma noradrenaline level was increased to 160.4 ± 13.1 pg/ml from the basal level of 104.2 ± 10.2 pg/ml ($n = 5$, $P < 0.05$) and plasma adrenaline level was increased to 348.0 ± 30.1 pg/ml from the basal level of 124.2 ± 24.6 pg/ml ($n = 5$, $p < 0.05$). When hexamethonium 10 mg/kg, a dose which is enough to block nicotinic receptors of the autonomic ganglia completely[22], was injected intraperitoneally between the first and second conditioned stimulus, the conditioned pressor response was almost abolished ($n = 5$, data not shown).

Intracerebroventricular injection of hexamethonium (1–10 µg) suppressed the pressor response elicited by the conditioned stimulus in a dose-dependent manner (fig. 2). Intraventricular injection of pentolinium (10 µg), another nicotinic receptor antagonist, also suppressed the conditioned pressor response (fig. 3). Hexamethonium or pentolinium did not affect the basal blood pressure. To examine whether those nicotinic receptor antagonists acted by blocking the peripheral

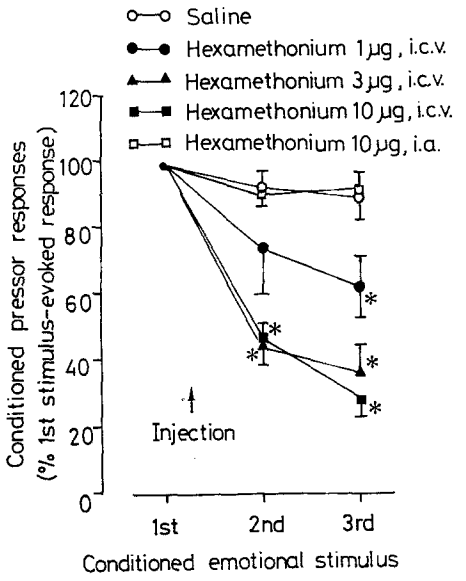

Figure 2. Effects of intracerebroventricular (i.v.c.) and intraarterial (i.a.) administration of hexamethonium on the mean blood pressure response to the conditioned emotional stimulus. Saline 10 µl (n = 7), hexamethonium 1 µg (n = 5), 3 µg (n = 5) or 10 µg (n = 6) was injected intraventricularly, and hexamethonium 10 µg (n = 5) was injected intraarterially between the first and second conditioned stimulus. Values are the mean ±SEM. *Significantly different from saline control, $p < 0.05$.

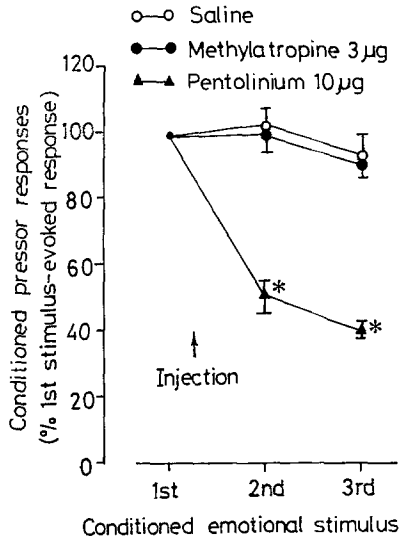

Figure 3. Effects of intracerebroventricular administration of saline 10 µl (n = 8), pentolinium 10 µg (n = 5), or methylatropine 3 µg (n = 6) on the mean blood pressure responses to the conditioned emotional stimulus. Drugs were injected between the first and second conditioned emotional stimulus. Values are the mean ±SEM. *Significantly different from saline control, $p < 0.05$.

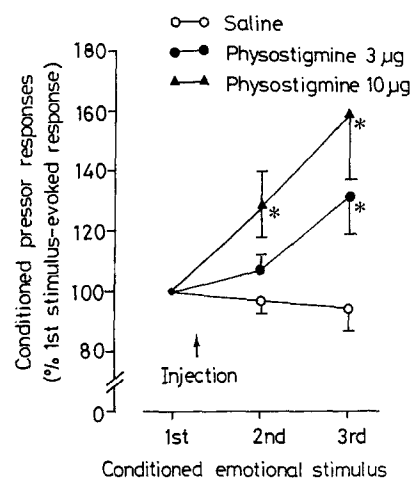

Figure 4. Effects of intracerebroventricular administration of saline 10 µl (n = 7), physostigmine 3 µg (n = 5) or 10 µg (n = 7) on the mean blood pressure responses to the conditioned emotional stimulus. Drugs were injected between the first and second conditioned emotional stimulus. Values are the mean ±SEM. *Significantly different from saline control, $p < 0.05$.

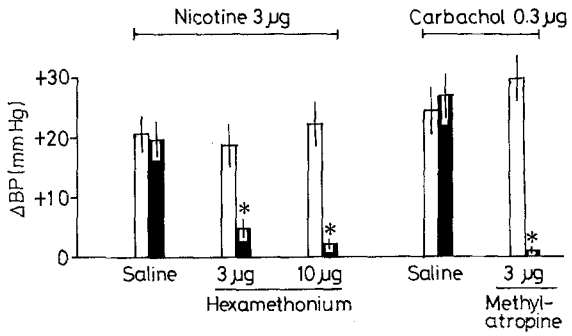

Figure 5. Effects of intracerebroventricular administration of saline, hexamethonium and methylatropine on the increase in mean blood pressure (BP) induced by intraventricular injection of nicotine (the first 6 columns) or carbachol (the last 4 columns). To determine effects of saline, hexamethonium and methylatropine, they were administered 10–15 min before nicotine or carbachol administration. Values are the mean ±SEM from 5 experiments. *Significantly different from before antagonist, $p < 0.05$ (paired t-test). Open columns and closed columns are respectively before and after saline, hexamethonium and methylatropine.

autonomic ganglia after leaking into the systemic circulation from the brain, we injected hexamethonium peripherally. Hexamethonium when injected intraarterially at 10 µg, a dose which was used in the intraventricular injection experiments, did not affect the conditioned pressure response (fig. 2), indicating that the effect of intraventricular injection of hexamethonium is a central one. The intraarterial hexamethonium did not affect basal blood pressure.

In contrast, intraventricular injection of methylatropine (3 µg), a muscarinic receptor antagonist, did not affect either the basal blood pressure or the pressor response elicited by the conditioned stimulus (fig. 3). Intraventricular injection of physostigmine (3 and 10 µg), a cholinesterase inhibitor, enhanced the conditioned pressor response in a dose-dependent manner (fig. 4). Although intraventricular (ICV) physostigmine (10 µg) produced an increase in basal blood pressure (20.0 ± 2.6 mm Hg, n = 7), the blood pressure returned to around pre-injection levels 15 min after injection.

The conditioned stimulus produced the species-typical response of rodents, crouching or freezing, which is thought to be an index of conditioned fear. The conditioned freezing lasted for 30 s during the conditioned stimulus. After the intraventricular injection of hexamethonium (10 μg), pentolinium (10 μg) and physostigmine (3 and 10 μg), the conditioned freezing response still lasted during the 30 s conditioned stimulus.

Finally, we examined whether those doses of hexamethonium and methylatropine used in this study indeed can block central nicotinic and muscarinic receptors, respectively. Intraventricular injection of the nicotinic receptor agonist nicotine (3 μg) produced an increase in blood pressure. Pretreatment with intraventricular hexamethonium (3 and 10 μg) inhibited the pressor response to nicotine in a dose-dependent manner (fig. 5). Intraventricular injection of carbachol (0.3 μg), a muscarinic receptor agonist, also produced an increase in blood pressure and this pressor response was completely inhibited by pretreatment with intraventricular methylatropine (3 μg).

Discussion

In the present study the conditioned stimulus previously paired with electric footshock consistently elicited an increase in blood pressure, which is a pure reflection of the emotional reaction to the conditioned stimulus rather than to the interaction of the conditioned stimulus and the unconditioned stimulus. The pressor response elicited by the conditioned stimulus was reproducible when applied three times every 20 min. Thus, this method allowed us to evaluate effects of drugs on emotionally conditioned pressor responses. The pressor response elicited by the conditioned stimulus was markedly inhibited by peripheral administration of hexamethonium (10 mg/kg, i.p.), and plasma noradrenaline and adrenaline levels were increased during the conditioned stimulus, suggesting that sympathetic nerves and adrenal medulla contribute to the conditioned pressor response. These results are consistent with those of the study by Sakaguchi et al.[23] who examined the effects of adrenal demedullation and chemical sympathectomy.

The nicotinic receptor antagonist hexamethonium injected intracerebroventricularly produced an inhibition of the pressor response elicited by the conditioned stimulus. The inhibitory effect of hexamethonium on the conditioned pressor response was parallel to that of hexamethonium on the pressor response to nicotine. Pentolinium, another nicotinic receptor antagonist, also inhibited the conditioned pressor response. On the other hand, intraarterial administration of hexamethonium, at the same dose (10 μg) used in the intraventricular injection experiments, did not affect the pressor response, indicating that the effect of intraventricular injection of hexamethonium is a central one, but not a peripheral one after the escape of hexamethonium from the brain into the systemic circulation. These findings are consistent with the hypothesis that central nicotinic receptors are involved in mediating the pressor response elicited by the conditioned stimulus.

In contrast, the muscarinic receptor antagonist methylatropine did not affect the pressor response elicited by the conditioned stimulus. The dose of atropine used in this study was enough to block central muscarinic receptors, since methylatropine (3 μg) produced complete blockade of the pressor response to carbachol, a muscarinic cholinergic agonist. Thus, we could not find involvement of central muscarinic receptors in mediating the conditioned pressor response.

The finding that blockade of central nicotinic receptors resulted in an inhibition of the conditioned pressor response suggests that endogenous brain acetylcholine has a role in the response. In the present study, indeed, the cholinesterase inhibitor physostigmine enhanced the pressor response elicited by the conditioned stimulus, which is consistent with this idea. It has been reported that acetylcholine release, acetylcholine content and choline acetyltransferase activity in the brain are increased following chronic stress[3–5].

In the present study, hexamethonium and pentolinium did not affect the conditioned fear response. Thus, it seems unlikely that the inhibitory effect of these nicotinic receptor antagonists on the conditioned pressor response results primarily from a reduction in emotional response to the conditioned stimulus. Further studies will be needed to clarify the exact mechanisms of the action of nicotinic receptor antagonists.

In summary, the pressor response elicited by the conditioned stimulus was inhibited by intraventricular injection of hexamethonium and pentolinium but not of methylatropine. Physostigmine enhanced the conditioned pressor response. These findings are consistent with the possibility that central nicotinic receptors are involved in mediation of the conditioned pressor response, and endogenous brain acetylcholine may itself play a role in this conditioned pressor response.

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