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EFFECTS OF CHOLINERGIC DRUGS AND THEIR BLOCKERS ON ADRENALINE RELEASE FROM RAT ADRENAL

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Abstract—Effects of cholinergic agents and their blockers on adrenaline secretion from rat adrenal were investigated. Nicotine-induced adrenaline output was markedly prevented by hexamethonium. High doses of atropine (1·0 mg/kg, i.v.) also decreased nicotine-induced adrenaline secretion from splanchnicotomized adrenal. Acetylcholine (ACh) subsequent to neostigmine caused marked secretion of adrenaline, which was almost completely blocked by atropine pretreatment. However, ACh-induced adrenaline output from the splanchnicotomized adrenal was much less than that from the intact gland. The response of chronically and completely denervated adrenal to ACh was larger than that of the chronically or acutely splanchnicotomized gland. Pilocarpine increased adrenaline secretion and the output was prevented by pretreatment with atropine. In acutely splanchnicotomized rats, pilocarpine caused little increase of adrenaline secretion. Atropine (1·0 mg/kg, i.p.) caused a slight increase of adrenaline output in response to ether anesthesia, although methylatropine tended to inhibit output. Stimulation of the central end of the severed vagus at the cervical level had no effect on adrenaline liberation from splanchnicotomized adrenal with or without stimulation of splanchnic nerve. From these results, the sites of action of these drugs in rats are discussed.

Many studies on adrenaline secretion from the adrenal medulla have been made on cats and dogs [1, 2]. However, similar studies on the rat, although it is one of the most popular experimental animals, are still lagging behind. In a previous paper, the author reported a method for studying the effect of drugs on adrenaline secretion from rat adrenal gland [3], and studies using this method have been performed on the effects of histamine, bradykinin and morphine [4], and on the nature of splanchnic-adrenal transmission [5]. The present paper demonstrates the action of cholinergic drugs and their blockers on adrenaline secretion in rats and discusses their sites of action.

MATERIALS AND METHODS

Experiments were carried out with male rats of the Sprague–Dawley strain weighing 400–600 g. Details of the methods used were given in a previous paper [3, 5], so only a brief explanation will be given here.

Adrenal-venous blood was generally collected for 15 min under pentobarbital sodium anesthesia or with ether inhalation, and adrenaline content in the blood specimen was determined fluorometrically. Splanchnicotomy was performed as follows: the left greater splanchnic nerve was incised near the diaphragm either 25 min prior to the onset of blood collection (acute) or a week prior to the experiment (chronic). Complete adrenal denervation was performed a week before intended use. In addition to splanchnicotomy near the diaphragm, the nerves were cut around the cardiac and coeliac ganglions. Vagotomy at the cervical level was performed just before abdominal operation. For vagal stimulation, a 5-min period of rectangular pulses (1 msec duration, 5 V and 20 c/sec) was applied 5 min after a conditioning tetanus. Hemorrhage was performed as follows: the prescribed volume of blood was withdrawn over a few min from the descending aorta by syringe.

The significance of the difference between the means of the responses was evaluated by Student's *t*-test.

The drugs used were nicotine (Tokyo Kasei), acetylcholine chloride (ACh; Kanto), neostigmine (Shionogi), pilocarpine hydrochloride (pilocarpine; Torii), hexamethonium bromide (C6; Yamanouchi), atropine sulfate (atropine; Merck) and atropine methylnitrate (methylatropine; Merck). These drugs were dissolved in 0·9% saline just before use and 0·5 ml/kg of the solutions were injected into the saphenous vein or i.p.

RESULTS

Nicotine. Hypersecretion of adrenaline caused by nicotine was markedly blocked by pretreatment with 0·25 to 1·0 mg/kg of C6 (Table 1). Atropine, in a high dose of 1·0 mg/kg, also inhibited the nicotine-induced hypersecretion of adrenaline from acutely splanchnicotomized adrenal gland (Table 2).

Acetylcholine. Infusion of ACh into the saphenous vein caused a slight increase of adrenaline secretion which was not significant at $P < 0·05$ (Fig. 1). In neostigmine-pretreated rats, ACh caused marked adrenaline secretion (Fig. 2). This hypersecretion of adrenaline was blocked almost completely by pretreatment with atropine or methylatropine (Table 3). However, adrenaline secretion from the splanchnicotomized adrenal caused by ACh subsequent to neostigmine was much less than that from the intact

Table 1. Effect of hexamethonium pretreatment on adrenaline release from rat adrenal in response to nicotine under pentobarbital anesthesia*

Hexamethonium bromide (mg/kg, i.v.)	Nicotine (mg/kg, i.v.)	N	Adrelaine output (left) (μg/15 min/kg)
0	0	13	0·04 ± 0·00
0	0·5	6	0·30 ± 0·06†
1	0·5	5	0·05 ± 0·02‡
0·25	0·5	5	0·08 ± 0·01†,§

* Hexamethonium was administered 5 min before nicotine injection. Data represent the mean ± S.E.
† Significantly different from untreated control group at $P < 0·01$.
‡ Significantly different from nicotine-treated group at $P < 0·01$.
§ Significantly different from nicotine-treated group at $P < 0·05$.

Table 2. Effect of atropine pretreatment on adrenaline release from acutely splanchnicotomized rat adrenal in response to nicotine under pentobarbital anesthesia*

Atropine sulfate (mg/kg, i.v.)	Nicotine (mg/kg, i.v.)	N	Adrenaline output (left) (μg/15 min/kg)
0	0·5	9	0·14 ± 0·03
1·0	0·5	5	0·07 ± 0·01†
0·2	0·5	5	0·11 ± 0·03

* Atropine was administered 5 min before nicotine injection. Data represent the mean ± S.E.
† Significantly different from nicotine only at $P < 0·05$.

Table 3. Effect of atropine and methylatropine on adrenaline release from rat adrenal in response to acetylcholine subsequent to neostigmine under pentobarbital anesthesia*

Treatment	N	Adrenaline output (left) (μg/15 min/kg)
Acetylcholine	6	0·92 ± 0·24
Atropine + ACh	6	0·11 ± 0·03†
Methylatropine + ACh	5	0·02 ± 0·01†,‡

* Neostigmine (0·2 mg/kg, i.v.) was administered 5 min before acetylcholine Cl injection (12·5 μg/kg, i.v.). Atropine sulfate (1·0 mg/kg, i.v.) and atropine methylnitrate (1·0 mg/kg, i.v.) were administered after the neostigmine injection. Data represent the mean ± S.E.
† Significantly different from acetylcholine only at $P < 0·05$.
‡ Significantly different from atropine + ACh at $P < 0·05$.

Table 4. Effect of denervation on adrenaline release from rat adrenal in response to acetylcholine subsequent to neostigmine under pentobarbital anesthesia*

Treatment	N	Adrenaline output (left) (μg/15 min/kg)
None (control)	6	0·92 ± 0·24
Acute splanchnicotomy	5	0·10 ± 0·05†
Chronic splanchnicotomy	6	0·17 ± 0·06†
Chronic complete adrenal denervation	7	0·45 ± 0·12

* Neostigmine (0·2 mg/kg, i.v.) was administered 5 min before injection of acetylcholine chloride (12·5 μg/kg, i.v.). Data represent the mean ± S.E.
† Significantly different from control at $P < 0·05$.

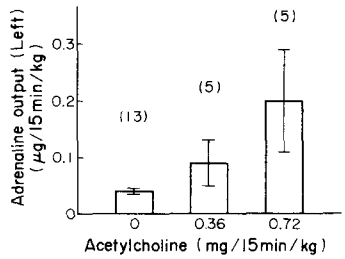

Fig. 1. Effect of acetylcholine on adrenaline release from rat adrenal under pentobarbital anesthesia. Acetylcholine was infused into the saphenous vein. The bars represent the mean and the vertical lines are S.E. Numbers of experiments are in parentheses.

adrenal. The response of the chronically splanchnicotomized adrenal to ACh was a little larger than that of the acutely splanchnicotomized gland; an even larger response was seen in the completely denervated gland (Table 4).

Pilocarpine. The effect of pilocarpine on adrenaline secretion from the rat adrenal is shown in Fig. 3. Increase of adrenaline secretion from the gland was observed after pilocarpine injection, but after pretreatment with 1·0 mg/kg of atropine, the pilocarpine-induced adrenaline secretion was markedly blocked. In acutely splanchnicotomized rats, little increase of adrenaline output was detected after 1·0 mg/kg of pilocarpine.

Atropine and methylatropine. The effects of atropine and methylatropine on adrenaline secretion from the adrenal gland caused by ether anesthesia are indicated in Fig. 4. The drugs were injected i.p. 10 min prior to ether anesthesia. Atropine, at a dose of 1·0 mg/kg, caused a slight increase of adrenaline output in response to ether anesthesia, but at a high dose (10 mg/kg) it caused slight inhibition, which was not significant at $P < 0.05$. Methylatropine, at doses of 0·25 to 1·0 mg/kg, tended only to inhibit output. Table 5 shows the effect of atropine on adrenaline liberation in response to cervical vagotomy. Bilateral vagotomy at the cervical level caused enhancement

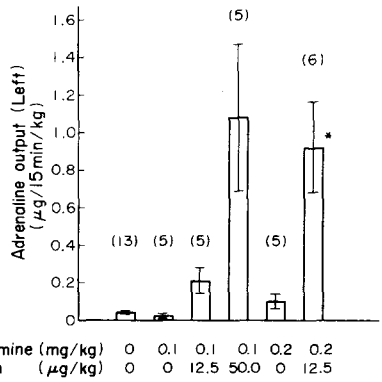

Fig. 2. Effect of acetylcholine on adrenaline release from rat adrenal pretreated with neostigmine under pentobarbital anesthesia. Neostigmine was administered 5 min before acetylcholine injection. The bars represent the mean and the vertical lines are S.E. Numbers of experiments are in parentheses. * Significantly different from control at $P < 0.05$.

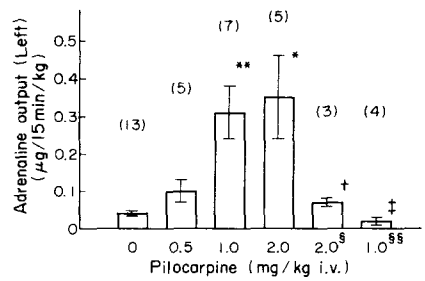

Fig. 3. Effect of pilocarpine on adrenaline release from rat adrenal under pentobarbital anesthesia. The bars represent the mean and the vertical lines are S.E. Numbers of experiments are in parentheses. § Atropine (1·0 mg/kg, i.v.) was administered 5 min before pilocarpine injection. §§ Acutely splanchnicotomized rats were used. * Significantly different from control at $P < 0.05$. ** Significantly different from control at $P < 0.01$. † Significantly different from the pilocarpine 1·0 mg/kg group at $P < 0.05$. ‡ Significantly different from the pilocarpine 1·0 mg/kg group at $P < 0.01$.

of adrenaline secretion under ether anesthesia, but monolateral vagotomy did not. Atropine (1·0 mg/kg), injected i.p. 10 min prior to ether anesthesia, did not produce further enhancement of adrenaline secretion caused by bilateral vagotomy.

Electrical stimulation of vagus. Stimulation of the central end of the severed vagus at the cervical level did not influence adrenaline secretion from splanchnicotomized adrenal with or without stimulation of splanchnic nerve (Tables 6 and 7).

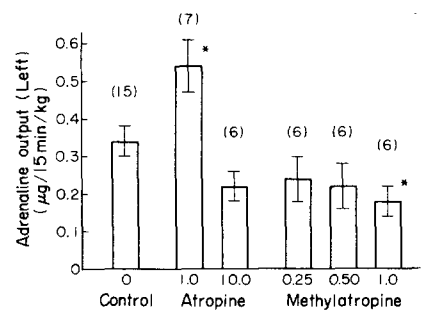

Fig. 4. Effect of atropine and methylatropine on adrenaline release from rat adrenal in response to ether anesthesia. Drugs (mg/kg) were administered i.p. 10 min before onset of ether anesthesia. The bars represent the mean and the vertical lines are S.E. Numbers of experiments are in parentheses. * Significantly different from control at $P < 0.05$.

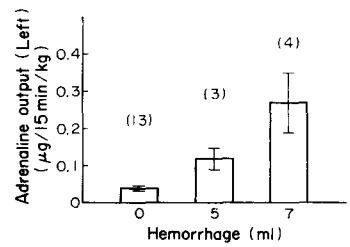

Fig. 5. Effect of hemorrhage on adrenaline release from rat adrenal under pentobarbital anesthesia. Blood was withdrawn over a few min from the descending aorta by syringe. The bars represent the mean and the vertical lines are S.E. Numbers of experiments are in parentheses.

Table 5. Effect of vagotomy on adrenaline release from rat adrenal under ether anesthesia*

Treatment	N	Adrenaline output (left) (μg/15 min/kg)
Control (sham)	6	0·45 ± 0·05
Vagotomy (bilateral)	6	0·94 ± 0·10†
Atropine‡ + vagotomy (bilateral)	6	0·81 ± 0·11§
Control (sham)	5	0·25 ± 0·04
Vagotomy (monolateral)	5	0·32 ± 0·10

* Vagotomy was performed at the cervical level just before abdominal operation. Data represent the mean ± S.E.
† Significantly different from control at $P < 0·01$.
‡ Atropine (1·0 mg/kg) was injected i.p. 10 min prior to ether anesthesia.
§ Significantly different from control at $P < 0·05$.

Hemorrhage. The influence of hemorrhage on adrenaline secretion is shown in Fig. 5. Removal of 5 and 7 ml of blood caused a slight or moderate increase in adrenaline output from the gland.

DISCUSSION

Rohr [6], in a histochemical study on rat adrenals, demonstrated that although ACh alone does not cause catecholamine release, after pretreatment with neostigmine, it causes marked hypersecretion of catecholamines. In our experiments, when ACh alone was infused through the saphenous vein, adrenal response was found, but the response was small and variable. After acetylcholinesterase had been inhibited by pretreatment with neostigmine, ACh caused marked adrenaline secretion from the adrenal gland. These results are biochemical evidence to support the histochemical findings of Rohr [6]. Feldberg and Minz [7] reported that ACh injected into atropinized cats, dogs and rabbits causes hypersecretion of adrenaline, and its secretory action is due to a direct effect upon the gland, and not to central or sympathetic stimulation. Yamashita *et al.* [8] showed that adrenaline secretory action of ACh is not inhibited, even by large doses of atropine, in anesthetized dogs. In rats, however, the action of ACh subsequent to neostigmine was markedly blocked by atropine, methylatropine and splanchnicotomy. Therefore, the action of ACh was mainly, directly or indirectly, due to its effect on the central nervous system (CNS) and not to direct action on the adrenal medulla. Similar results were observed for the effect of pilocarpine. Trendelenburg [9] reported that a fall of blood pressure through severe bleeding causes an increase in adrenaline concentration but not in actual output. Saito [10] demonstrated that hemorrhage evokes an increase, though slight, of the adrenaline discharge in dogs. In rats too, adrenaline release caused by bleeding was not large. Possible hypotension-induced adrenaline

Table 6. Effect of electrical stimulation of both vagi on adrenaline release from splanchnicotomized rat adrenal under pentobarbital anesthesia*

Treatment	N	Adrenaline output (left) (μg/10 min/μg)
Control	4	0·06 ± 0·02
Vagus stimulation	4	0·04 ± 0·01

* A 5-min period of rectangular pulses (1 msec duration, 5 V and 20 c/sec) was applied 5 min after a conditioning tetanus. Data represent the mean ± S.E.

Table 7. Effect of electrical stimulation of both vagi on adrenaline release from rat adrenal in response to electrical stimulation of splanchnic nerve under pentobarbital anesthesia*

Treatment	N	Adrenaline output (left) (% of control)
Vagus stimulation	6	100·8 ± 5·6

* Two trains of tetanus (fifteen 10-sec rectangular pulses, 1 msec, 5 V, 20 c/sec, at 10-sec intervals) were applied to the left splanchnic nerve at a 5-min interval in the same animal. Stimulation (1 msec, 5 V, 20 c/sec) of vagus was applied simultaneously with one of the splanchnic nerve stimulation periods. Data represent the mean ± S.E. Control value was 1·39 ± 0·17 μg.

output after ACh injection is not thought to be sufficiently large to account for the effect seen. It seems undeniable, therefore, that ACh, in this condition, increases adrenaline secretion through a direct action on the CNS.

ACh-induced adrenaline release from the completely denervated adrenal was greater than that from the gland splanchnicotomized near the diaphragm. Generally, the internal organs are innervated with sympathetic nerves and vagi. If the vagi are the inhibitory nerves, their removal may be thought to be a cause of this phenomenon. However, there has been little evidence that the vagi peripherally control adrenaline secretion from the adrenal medulla [11]. In our experiments, electrical stimulation of the vagus sectioned at the cervical level caused no influence on adrenaline secretion from the splanchnicotomized adrenal, with or without stimulation of the splanchnic nerve. Therefore, the difference in the adrenal response to ACh by different denervation methods may be due to differences in the degree of denervation hypersensitivity.

It has been reported that C6 inhibits adrenal medullary response to adrenaline secretagogues, and that this inhibition is due to paralysis of the adrenal medulla [12–14]. In our experiments, C6 and atropine diminished the adrenaline secretion in response to nicotine. Fink and Cervoni [15] showed that atropine and methylatropine have ganglionic blocking actions in cats; the action of the latter is much stronger than that of the former, but both are much weaker than C6. The inhibitory effect of atropine on nicotine-induced adrenaline secretion from the acutely splanchnicotomized adrenal may be due to its blocking action on nicotinic receptors. The stronger blockade of ACh-induced adrenaline secretion by methylatropine could be explained by a difference in the strength of ganglionic blocking actions. Atropine in a low dose enhanced and in a high dose diminished the adrenaline secretion caused by ether anesthesia, while methylatropine produced only inhibition of adrenaline secretion. In dogs too, Sato *et al.* [16] demonstrated that atropine evoked an acceleration in the adrenaline output rate. Ether causes adrenaline hypersecretion through excitation of the splanchnic nerve [3] and atropine has an inhibitory action on adrenaline release caused by electrical stimulation of this nerve sectioned near the diaphragm [5]. It is concluded that the enhancement of ether-induced adrenaline secretion by atropine is due to its effect on the CNS. This conclusion is supported by the fact that atropine has an effect on the CNS, while methylatropine does not [17]. Tigyi *et al.* [18] reported that adrenaline output increases after bilateral cervical vagotomy in dogs and suggested that the site of action may be the parasympathetic region of the hypothalamus. In our experiments, bilateral cervical vagotomy caused enhancement of adrenaline secretion under ether anesthesia, and atropine did not cause further enhancement of this output. These results suggest that the site of action of atropine on adrenaline hypersecretion is the parasympathetic region, giving the same effect as bilateral vagotomy.

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