EFFECT OF 4-AMINOPYRIDINE ON THE RAT SUPERIOR CERVICAL GANGLION*

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Abstract—Effect of 4-aminopyridine (4-AP) on the isolated superior cervical ganglion of the rat was investigated by means of the sucrose-gap-method. 4-AP initiated discharges of postganglionic neurons and simultaneously increased the amplitude of the compound action potential evoked by supra-maximal preganglionic nerve stimulation at concentrations higher than 0.1 mM. Ganglionic discharges induced by 4-AP were characterized by low frequency and high amplitude. These 4-AP-induced discharges were unaffected by removal of the preganglionic nerve trunk. These discharges were suppressed by d-tubocurarine, Ca removal from saline, or preganglionic denervation, whereas discharges of high frequency and low amplitude were observed after 4-AP in Ca-free solution or in denervated preparations. During perfusion with 4-AP, antidromic action potentials were recorded from the preganglionic nerve. When 4-AP was applied to the preganglionic nerve trunk, however, postganglionic responses were unaffected. It is suggested that discharges of postganglionic neurons were induced by 4-AP, possibly as a result of an initiation of action potentials in preganglionic terminal fibres running in the ganglion.

4-Aminopyridine (4-AP) has been found to selectively block the K^+ conductance in several excitable tissues. In the squid giant axon, 4-AP reduces both inward- and outward-going K^+ currents, whereas tetraethylammonium (TEA) blocks only the outward movement of K ions (1-3). 4-AP depolarizes the squid axon membrane and slightly prolongs the duration of the action potential. These effects of 4-AP are reversed by higher depolarization (1-3). It has also

been observed that the action of 4-AP is more selective for the K^+ current as compared with the action of TEA on the cockroach giant axon (4) and skeletal muscle fibres (5, 6). In the neuromuscular junction, 4-AP prolongs the duration of the presynaptic action potential by inhibition of the K^+ conductance (7) and is suggested to enhance the influx of Ca ions during depolarization of the nerve terminal (8-10). An increase in the concentration of Ca ions in the presynaptic terminal may lead to facilitation of transmitter release from the nerve terminal, and consequently muscle contractility may be increased by 4-AP. Similar phenomena have also been reported in the smooth muscle neuroeffector junction (11).

Though it has been demonstrated that

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4-AP exerts profound effects on some excitable membranes, the effect of 4-AP on mammalian sympathetic ganglion has not been reported as yet. In the course of a study on the effect of the gK blockers on the isolated rat superior cervical ganglion, we found that 4-AP generated repetitive discharges in the postganglionic neurons which were characterized by low frequency and high amplitude and these were quite different from discharges induced by hitherto known nicotinic or muscarinic ganglion stimulants. The latter discharges are characterized by higher frequency and lower amplitude (12–15) than the 4-AP-induced discharges. In the present study, we examined the nature of these discharges and subsequently explored where the discharges originated.

MATERIALS AND METHODS

Preparation: Superior cervical ganglia with pre- and post-ganglionic nerve trunks were excised from male Wistar rats weighing 450–550 g. The ganglia were immersed in the physiological saline, and the connective tissue sheath was carefully removed under a binocular microscope. Thereafter, the preparation was placed in a perfusion chamber and perfused continuously (approximately 0.5 ml/min) with saline, to which the drugs to be tested were added.

The physiological saline had the following composition in mM: NaCl, 137.9; KCl, 4.0; CaCl₂, 2.0; MgCl₂, 0.5; NaHCO₃, 12.0; KH₂PO₄, 1.0; glucose, 11.1. The solution was gassed with 5% CO₂ and 95% O₂. Calcium-free saline was prepared by replacing CaCl₂ with MgCl₂. All experiments were carried out at about 30°C.

Recording of electrical activities: A modified version of Nishi and Koketsu's sucrose-gap arrangement (16) was used for recording the changes of membrane potentials of ganglion cells or preganglionic nerve fibres. Membrane potentials were fed via

calomel-wick electrodes to an amplifier which consisted of operational amplifiers, LF356 and μ A741. Ganglionic or preganglionic action potentials were displayed on an oscilloscope (model VC-7, Nihon-Kohden Ltd.), and the changes of membrane potential were displayed on a DC pen-recorder (EPR-2TB, Toa Electronics). In most preparations, a resting membrane potential of about 30 mV was obtained.

Preganglionic denervation: In some rats the preganglionic nerve was sectioned unilaterally 10 to 14 days before experiment under thiopental anaesthesia, and the animals were allowed to recover. Contralateral ganglia served as controls.

Drugs used: The following compounds were used: 4-aminopyridine and d-tubocurarine chloride (both from Wako Pure Chemicals) and 1,1-dimethyl-4-phenylpiperazinium iodide (Tokyo Kasei).

RESULTS

Effect of 4-AP on the compound action potential: The preganglionic nerve was stimulated supramaximally, and the evoked compound action potential (17) was recorded from the postganglionic neurons. When ganglia were perfused with 0.5 mM 4-AP for 5 to 10 min, the amplitude of this action potential was markedly increased (Fig. 1A), and the mean membrane potential of the postganglionic neurons was gradually decreased (Fig. 1C).

4-AP-induced ganglionic discharges: Spontaneous activity was not observed in the rat superior cervical ganglion under normal conditions (Fig. 1Ba). When ganglia were perfused with 0.5 mM 4-AP for 5 to 10 min, action potentials were induced in the postganglionic neurons (Fig. 1Bb), and the membrane potential was gradually decreased.

These 4-AP-induced ganglionic discharges were characterized by low frequency and high amplitude as compared with those induced by

ganglionic stimulants (12–15).

Dose-dependency of the effect of 4-AP:

In a concentration range of 0.01–1 mM, 4-AP augmented the compound action potential dose-dependently. Both frequency and amplitude of 4-AP-induced ganglionic dis-

charges were also increased in a similar way (Fig. 2A and B). Two preparations were used.

The following experiments were undertaken to determine the site of action involved in the generation of ganglionic action potentials by 4-AP.

Effect of d-tubocurarine (d-Tc) on the 4-AP-induced ganglionic discharges:

After the ganglionic transmission was completely blocked by an exposure to 1 mM d-Tc for 10 min, saline containing 1 mM 4-AP and 1 mM d-Tc failed to generate the ganglionic discharges (Fig. 3Bc), while membrane depolarization in the postganglionic neurons was observed (Fig. 3C).

Removal of external calcium ions:

Perfusion medium from which Ca ions were removed was utilized in an attempt to inhibit the release of transmitter from preganglionic nerve endings. When ganglia were perfused with Ca-free saline for 40 min, the compound action potential evoked by preganglionic nerve stimulation was abolished (Fig. 4Ab) and the membrane potential was slightly depolarized. Under these conditions, ganglionic discharges during perfusion of 0.5 mM 4-AP were apparently depressed, but discharges of high frequency and low amplitude and a gradual depolarization of the postganglionic neurons were recorded (Fig. 4Bc and C).

Fig. 1. Effects of 4-AP on compound action potential (A) and postganglionic neuronal activity (B). a: Control in normal saline. b: After 10 min perfusion with 0.5 mM 4-AP. C: Changes of the membrane potential during perfusion with 0.5 mM 4-AP. An upward deflection corresponds to depolarization. Recordings shown are typical of 11 for A and of 15 for B and C.

Fig. 2. Dose-dependency of responses to various concentrations of 4-AP. A: Compound action potentials. B: Postganglionic discharges. These results were obtained after 10 min perfusion with 4-AP at the concentrations of 0.01, 0.1, and 1 mM.

Effect of preganglionic denervation:

Denervated superior cervical ganglia were isolated with the scar of the degenerated preganglionic nerve. Stimulation of the scar and the neighboring area failed to evoke any compound action potential indicating successful denervation of the ganglion (Fig. 5Aa).

In 3 of 5 denervated ganglia, 0.5 mM 4-AP failed to generate any discharges of the postganglionic neurons, while in 2 ganglia discharges of high frequency and very low amplitude were observed (Fig. 5Bb). On the other hand, the membrane of the denervated ganglia was depolarized by 4-AP, and these ganglia responded to 0.05 mM dimethylphenylpiperazinium (DMPP) after 4-AP-perfusion to produce discharges of high frequency and low amplitude. Responses of contralateral ganglia to 4-AP were unaffected.

Fig. 3. Abolition of the compound action potential and the 4-AP-induced postganglionic discharges by d-tubocurarine. A: Compound action potential produced by preganglionic nerve stimulation. B: Postganglionic neuronal activity. a: After 10 min perfusion with 0.5 mM 4-AP. b and c: Immediately and 10 min after the perfusion with 0.5 mM 4-AP+1 mM d-Tc, respectively. C: Changes of the membrane potential during perfusion with 0.5 mM. Typical of 7 observations.

Fig. 4. Effect of 4-AP in Ca-free medium. A: Compound action potential produced by preganglionic nerve stimulation. B: Postganglionic neuronal activity. a: After 10 min perfusion with 0.5 mM 4-AP. b and c: Immediately and 10 min after Ca removal, respectively. C: Changes of the membrane potential during perfusion with 0.5 mM 4-AP in Ca-free medium. Typical of 9 observations.

Fig. 5. Effect of 4-AP on a denervated ganglion. A: Lack of response to the stimulation of the degenerated preganglionic nerve. B: Postganglionic neuronal activity. a: Control in the normal saline. b: After 10 min perfusion with 0.5 mM 4-AP. Fast and very small discharges are seen. C: Change in membrane potential during perfusion with 0.5 mM 4-AP in denervated ganglion. Typical of 5 observations.

Fig. 6. Effects of 4-AP on preganglionic axonal conduction (A) and axonal membrane activity (B). a and b: Before and after 10 min perfusion with 0.5 mM 4-AP. C: Changes of the membrane potential in the axonal part during perfusion with 0.5 mM 4-AP. Typical of 10 observations.

Effect of 4-AP on preganglionic axonal conduction and axonal membrane activity:

As 4-AP affects the excitable membrane extensively, axonal membranes of preganglionic nerve fibres were also regarded as one of the sites of action of 4-AP. When the preganglionic nerve trunk was perfused with 0.5 mM 4-AP for 5 to 10 min, the amplitude of the action potentials evoked by a single preganglionic volley conducting antidromically was obviously increased (Fig. 6Ab).

In most preparations, neither spontaneous activity nor any 4-AP-induced activity was observed (Fig. 6Ba). In a few (2 out of 10) preparations, action potentials which were dispersed and of low amplitude were recorded from the preganglionic axonal part. Axonal membrane potential tended to be reduced during the perfusion with 4-AP (Fig. 6C).

Effect of 4-AP applied to different sites:

When 10 ganglia were perfused with 0.5 mM 4-AP for 5 to 10 min, antidromically conducting action potentials were recorded from

Fig. 7. Antidromic action potentials in the preganglionic nerve terminals. The ganglion was perfused with 0.5 mM 4-AP, and the discharges in the preganglionic terminal fibres were recorded through the preganglionic nerve. The setting of the recording electrodes and the site of application of 4-AP are shown in the inset. Typical of 10 experiments.

the preganglionic terminal fibres. Amplitudes of these potentials were much lower than those of 4-AP-induced postganglionic discharges (Fig. 7). Depolarization was observed during the perfusion. These discharges were hardly affected by 1 mM dTc, which fully suppressed the 4-AP-induced discharges and ganglionic transmission.

In 3 preparations, the preganglionic nerve trunk was cut away at the central end of the ganglia in order to eliminate the possibility that 4-AP affected the preganglionic axons. The effect of 0.5 mM 4-AP was not changed by this dissection (Fig. 8A).

In other experiments using 2 preparations, 4-AP was applied locally to the preganglionic axonal part, and changes of the ganglionic responses were recorded from the postganglionic neurons. Perfusion with 0.5 mM 4-AP for 5 to 10 min did not affect the shape of the compound action potential (Fig. 8B). In the postganglionic neurons, discharges were not induced (Fig. 8C) nor was the resting potential affected.

DISCUSSION

4-AP depolarized the excitable membranes of pre- and post-ganglionic neurons in the rat superior cervical ganglion. In this respect, it appears that the properties of the neuronal membrane of the rat are similar to those of the

Fig. 8. Site of action of 4-AP in inducing the discharges of low frequency and high amplitude. A: Postganglionic neuronal activity of a ganglion from which preganglionic nerve had been removed. B and C: Effects of 4-AP applied to the preganglionic nerve trunk on the compound action potential and postganglionic neuronal activity, respectively. The setting of the recording electrodes and the site of application of 4-AP are shown in the insets.

squid giant axon (1–3), and membrane depolarization in ganglia is probably due to a decrease in K^+ conductance by 4-AP.

The compound action potential evoked by preganglionic nerve stimulation was enhanced in amplitude by 4-AP, but no distinct prolongation of the duration was observed, though some prolongation was expected. The mechanisms underlying the effect of 4-AP on the compound action potential are now still obscure and require further investigation.

Isolated ganglia of the rat did not exhibit spontaneous spike discharges normally, while repetitive action potentials were generated in the postganglionic neurons by an application of 4-AP. These 4-AP-induced ganglionic discharges were characterized by low frequency and high amplitude. On the other hand, ganglionic responses induced by nicotinic and muscarinic stimulants are usually characterized by high frequency and low amplitude (12–15) in contrast to the discharges induced by 4-AP. In our recording system, the amplitude of the acetylcholine-

induced discharges maximally attains a level of ca. 50–70 μV . From this point of view, it seemed that the generation of ganglionic discharges by 4-AP did not result from the possible direct nicotinic action of 4-AP on the postganglionic neuron. It is evident, however, that the nicotinic receptors were involved in the mechanisms of the generation of ganglionic discharges by 4-AP since 4-AP-induced ganglionic discharges were obviously depressed by d-Tc.

Many studies have demonstrated that 4-AP acts on the motor nerve terminals and facilitates neuromuscular transmission (7–10). Therefore, the possibility that 4-AP-induced ganglionic discharges were elicited by transmitter release from preganglionic nerve endings was suspected. It is known that in sympathetic ganglia, removal of Ca ions from the perfusion medium causes a synaptic blockade by abolition of transmitter release (18, 19). The 4-AP-induced ganglionic discharges were completely abolished under these conditions and also in the denervated ganglia. However, as discharges

of high frequency and low amplitude were produced in Ca-free medium by 4-AP and in denervated ganglia by an addition of DMPP after 4-AP, it is reasonable to consider that the ability of the cells to produce action potentials was retained in Ca-free medium or after denervation. These facts suggest that release of ACh is involved in the mechanisms of generation of the 4-AP-induced ganglionic discharges characterized by low frequency and high amplitude.

4-AP may act on the preganglionic nerve fibres and initiate action potentials in them. However, the sites where these action potentials were generated might be limited. Alternatively, 4-AP might release ACh without accompanying action potentials. When only the preganglionic nerve trunk was perfused with 4-AP, no ganglionic discharges were recorded, and antidromic axonal discharges were seldom recorded. In contrast, when only the ganglia were perfused with 4-AP, discharges of low frequency and high amplitude accompanied by membrane depolarization were recorded from the ganglia and antidromic discharges were recorded from the preganglionic nerves, indicating membrane activity of the intraganglionic terminal fibres. Moreover in the preparation from which preganglionic nerve trunk was removed at the proximal end of the ganglia, 4-AP-induced ganglionic discharges were recorded from postganglionic neurons. These results indicate that the origin of 4-AP-induced ganglionic discharges probably resides at the intraganglionic preganglionic nerves and/or nerve terminals, and it is suggested that the 4-AP-induced ganglionic discharges were a consequence of preganglionic action potentials initiated by the drug.

It has been reported that 4-AP induces a slow automatic activity in the squid giant axon (20) and generates antidromic action potentials presynaptically in the abdominal

ganglion of the cockroach (21). 4-AP has also been shown to generate a repetitive firing of autonomic nerve fibres (22). It seems likely that similar phenomenon was caused in the preganglionic nerves of the superior cervical ganglion of the rat.

The distinguishing characteristics of 4-AP-induced postganglionic discharges, low frequency and high amplitude, should be accounted for. One feature of the synaptic organization of the sympathetic ganglion is its divergence. Therefore, if an action potential is initiated at a site of the preganglionic nerve fibres by 4-AP, it will reach a number of nerve endings. Consequently, a number of postganglionic neurons innervated by these fibres will be simultaneously excited. These assumptions can tentatively explain the characteristics of the 4-AP-induced ganglionic discharges. The membrane mechanisms underlying the initiation of action potential by 4-AP, probably in the preganglionic nerve terminal within the ganglion, remain to be elucidated.

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