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ATRIOPEPTIN III DEPRESSES THE EXCITABILITY OF SYMPATHETIC NEURONES

Donald W. Cheung

Department of Pharmacology, University of Toronto,
Toronto, Ontario M5S 1A8, CANADA

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SUMMARY: The effect of atriopeptin III (AP III) on the excitability of the inferior mesenteric ganglion (IMG) of the guinea-pig was studied by intracellular recording technique. AP III depressed the excitability of the IMG by decreasing the frequency of the action potentials, prolonging the afterpotential and increasing the threshold of the action potentials. Voltage-clamp studies revealed that a transient potassium current - the A current - was enhanced by AP III. It is suggested that inhibition of the sympathetic nervous system may contribute to the cardiovascular effect of AP III.

Peptides isolated from mammalian atria, the atrial natriuretic factors (ANF), are known originally for their potent natriuretic and diuretic actions (1). Later studies indicate that they also have inhibitory effects on the vascular smooth muscle (2) and hormonal secretion (3-5). Recently, a possible functional role of ANF in the nervous system has been implicated. In the central nervous system, ANF-like immunoreactivity has been identified in the hypothalamus (6,7) and spontaneous firing of neurones in this region were inhibited by applied ANF (7,8). ANF-like immunoreactivity has also been identified in sympathetic neurones (9-11) but the physiological effects of ANF were not known. In the present study, we demonstrate that atriopeptin III (AP III) depresses the excitability of a sympathetic ganglion, the inferior mesenteric ganglion (IMG) of the guinea-pig. The inhibitory action of AP III is due to an increase in a transient outward potassium current, the I_A.

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METHODS

Male guinea-pigs 200-250 g were used in the study. The isolated IMGs were superfused with oxygenated physiological saline (in mM: NaCl 120; $NaHCO_3$ 25; $MgSO_4$ 1; KCl 5; $CaCl_2$ 2.5; NaH_2PO_4 1; glucose 11) at 37 C in a recording chamber. Electrical recordings were made using either single discontinuous current clamp or single electrode voltage clamp with an Axoclamp 2A (Axon Instruments). The micropipettes were filled with 3 M KCl and of 40 - 50 Mohms resistance. Electrical signals were stored on magnetic tapes and replayed on a Gould S2200 chart recorder. Recordings were taken only from cells where impalement was maintained throughout the experiment in order to eliminate variation between neurones. A concentration of 31 nM of AP III (Bio-Mega) was used in all experiments and recordings were made 15 to 30 minutes after the introduction of AP III. All values were expressed as mean $\pm$ standard error. Student's paired t-tests were used to compare the responses before and after AP III.

RESULTS

Action potentials were elicited when depolarizing currents were injected into the IMG neurones. Fig. 1a shows that a single action potential was triggered with a small depolarizing current. The most siginificant change following application of AP III (31 nM) was a slowing of the decay of the after-hyperpolarization. Whereas the recovery from the after-hyperpolarization was convex in shape in control, the depolarization became concave in the presence of AP III (Fig. 1a). The time interval between the peak after-hyperpolarization and the time for it to decay to half the ampltidue of the electrotonic potential (Fig. 1a, arrows) was 41.6 $\pm$ 8.2 ms before and increased

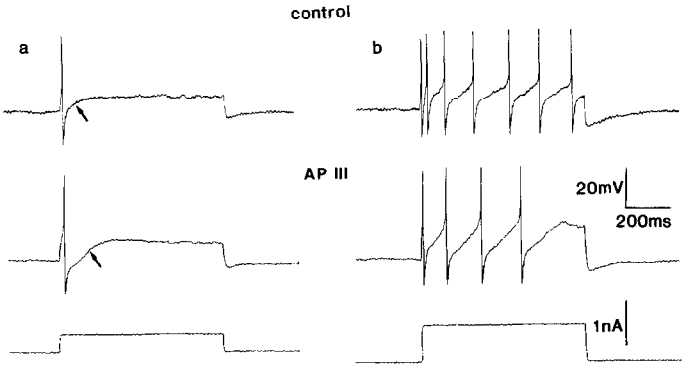

Fig. 1. The effects of AP III (31 nM) on the excitability of an IMG neurone. a. Action potential elicited by a depolarizing current (bottom trace) in the absence (top) and in the presence of AP III (middle). The arrows mark the half-amplitude of the electrotonic potentials. b. Multiple action potentials elicited by injection of a stronger depolarizing current.

significantly to 83.6 ± 8.6 ms after AP III (n = 7 preparations, $p < 0.01$). With increased depolarizing pulse, multiple action potentials were generated (Fig. 1b). The frequency of the action potentials was significantly decreased after the addition of AP III. In 5 preparations, the interval between the first and second action potentials was determined and was found to increase significantly ($p < 0.01$) from an average of 32.5 ± 4.8 ms to 128.3 ± 20.4 ms. In the control, a depolarization of 12.2 ± 0.8 mV was required to reach the threshold for the action potential. In the presence of AP III, the threshold is increased significantly to 15.4 ± 0.9 mV (n = 14 preparations, $p < 0.01$).

Similar to other sympathetic neurones, action potentials could also be generated by anodal-break excitation in some cells in the IMG. The hyperpolarization response of the IMG was not stable but relaxed towards the resting membrane potential. At the termination of the hyperpolarizing pulse, a transient depolarization that may lead to an action potential was observed (Fig. 2). With the addition of AP III, the transient depolarization and anodal-break excitation were suppressed. However, the relaxation of the membrane potential during the hyperpolarization was not affected.

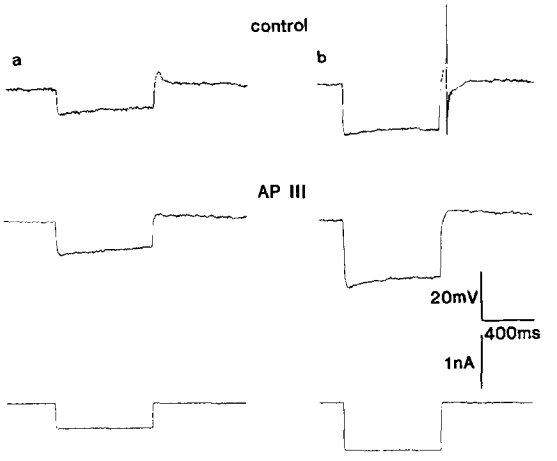

Fig. 2. Membrane responses to the application of hyperpolarizing currents (bottom traces) in control (top) and in the presence of AP III (31 nM) (middle). Rebound depolarization (a) and anodal-break excitation (b) were observed only in the controls.

The excitability of many neurones, including sympathetic neurones, is known to be regulated by two types of potassium currents - the M current, and the transient A current (12-15). Voltage clamp studies using a single electrode show that both currents are present in the IMG (12,16). In the same neurone as shown in Figs. 1 and 2, current relaxation reflecting the closing of M-channels was observed when the membrane was hyperpolarized (Fig. 3a). In eight preparations tested, the amplitude of the M-current did not alter after the addition of AP III. Following the hyperpolarization step, a small transient outward current resembling the I_A was generated. This transient outward current was enhanced in all eight preparations tested (Fig. 3).

DISCUSSION

The frequency of firing in many types of neurones is regulated by I_A. When I_A is blocked by 4-aminopyridine (15) or muscarinic agonists (12), the firing frequency is increased and the threshold decreased. Results from the present study showed that AP III enhanced the amplitude of I_A, thereby decreasing the frequency and increasing the threshold of the action potentials. The slowing of the decay of the after-hyperpolarization may also reflect enhanced activation of I_A. The voltage relaxation during hyperpolarization reflects the closing of M-

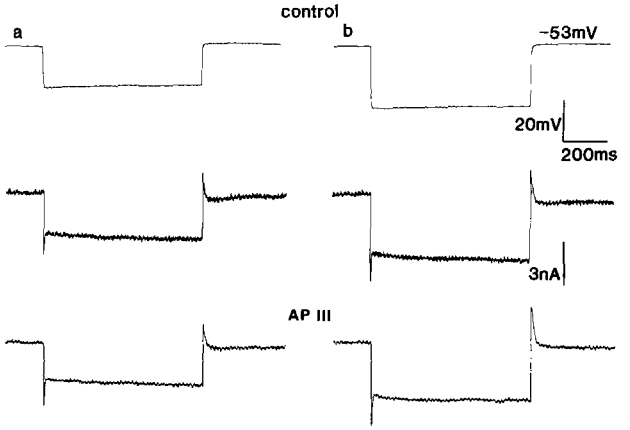

Fig. 3. Membrane currents in response to hyperpolarizing steps (top traces) of 20 mV (a) and 30 mV (b). Recordings were made in the same cell as in Figs. 1 & 2.

channels (14). This voltage response was not altered following AP III application, in agreement with the voltage clamp results. However, anodal-break excitation was abolished. This is compatible with the activation of an enhanced I_A following a hyperpolarization step. Thus the membrane effects of AP III on the IMG could be explained on the basis of an enhanced I_A.

Various studies have indicated that ANF may have physiological effects on the sympathetic nervous system. Thus an inhibitory effect on transmitter release from the sympathetic nerve endings by ANF has been demonstrated (17,18). A decrease in symathetic reflex activities following ANF-induced hypotension has also been shown (18,19). The present study shows that ANF can exert a direct action on the sympathetic neurones. With the finding of ANF-like immunoreactivities in sympathetic neurones, a physiological role of ANF in the modulation of neuronal activity is implicated.

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