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EFFECTS OF VASOPRESSIN ON SMOOTH MUSCLE CELLS OF GUINEA-PIG MESENTERIC VESSELS

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1 The effects of vasopressin on the membrane and contractile properties of smooth muscle cells of guinea-pig mesenteric arteries, and mesenteric and portal veins were investigated in various ionic environments by means of a micro-electrode technique and an isometric tension recording method. The results were compared with those obtained with oxytocin and noradrenaline (NA).

2 In the mesenteric jejunal artery, the mean membrane potential was -56.6 ± 2.3 mV, s.d, and the membrane was electrically quiescent. Application of outward current pulses generated small graded responses, and the current voltage relationship was linear with application of an inward current pulse.

3 Vasopressin and NA depolarized the membrane and increased the membrane resistance. Vasopressin was a 1000 times more potent than oxytocin in depolarizing the membrane. In high concentrations, vasopressin (1×10^{-3} or 1×10^{-2} iu/ml) or NA (5.9×10^{-5} M) generated slow oscillatory membrane potential changes (slow waves) and spikes during the depolarization. The excitatory actions of vasopressin and NA were not suppressed by tetrodotoxin (3.1×10^{-7} M) or ouabain (1.3×10^{-6} M) and the actions of vasopressin were not suppressed by adrenoceptor blocking agents (3.9×10^{-7} M phentolamine or 3.6×10^{-7} M propranolol).

4 The depolarization induced by vasopressin or NA is mainly due to a decrease in the K-permeability of the membrane. However, the contribution of other ionic species to the depolarization induced by vasopressin or NA differed, e.g. in low concentrations of $[Na]_o$, the NA-induced depolarization was suppressed to a greater extent than that due to vasopressin. In low concentrations of $[Ca]_o$, the vasopressin-induced depolarization was suppressed to a greater extent than with NA.

5 In low concentrations of $[Ca]_o$ and in the presence of vasopressin or NA, spike generation was inhibited but slow waves were not. In low concentrations of $[Na]_o$, the vasopressin-induced slow waves and spikes were for the great part preserved, but with a high concentration of $[Ca]_o$, vasopressing-induced slow waves were suppressed.

6 Both vasopressin and NA produced contractions in the jejunal mesenteric artery. However, the maximum contraction in response to vasopressin was larger than that to NA, although both induced similar membrane depolarization. In a low concentration of $[Na]_o$, vasopressin but not NA produced a contraction.

7 In the cranial mesenteric artery, NA (5.9×10^{-5} M) depolarized the membrane and produced a contraction, while vasopressin (1×10^{-1} iu/ml) and oxytocin (1×10^{-1} iu/ml) neither depolarized the member nor produced a contraction. In the mesenteric vein, NA (5.9×10^{-5} M) slightly depolarized the membrane and produced a small contraction. On the other hand, in the portal vein, NA (5.9×10^{-7} M) produced a marked depolarization and a contraction. Vasopressin (1×10^{-1} iu/ml) and oxytocin (1×10^{-1} iu/ml) produced neither excitatory nor inhibitory actions in these veins.

8 It is concluded that vasopressin acts on only small muscular arteries, while NA acts on all mesenteric vessels, particularly the portal vein. Therefore, the hepatic portal vascular resistance may be increased by NA and reduced by vasopressin.

Introduction

Vasopressin has a potent vasopressor effect physiologically (Cowley, Monos & Guyton, 1974) and pathophysiologically (Rocha e Silva & Rosenberg, 1969). In fact, it has a potent systemic vasoconstrictor action enabling it to make a significant contribution to the restoration of arterial pressure during haemorrhage (Laycock, Penn, Shirley & Walter, 1979; Cowley, Switzer & Guinn, 1980), though severe vasoconstriction of the mesenteric vascular bed may be responsible for irreversible haemorrhagic shock

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(Errington & Rocha e Silva, 1974). Furthermore, vasopressin is widely used for the treatment of massive oesophageal vascular haemorrhage. Its main therapeutic mechanism is explained by a reduction of portal blood flow with a fall in hepatic portal venous pressure due to mesenteric vasoconstrictor activities (Richardson & Withrington, 1978). Since vasopressin reduced the hepatic portal vascular resistance and NA increases it (Richardson & Withrington, 1977), the fall in hepatic portal venous pressure may be characteristic of vasopressin. In *in vivo* experiments, by direct microscopic observation, Altura (1973) found that vasopressin constricts mesenteric arteries, arterioles and venules. In *in vitro* experiments, vasopressin was shown to produce a contraction of the mesenteric artery (Altura, 1970), while this hormone inhibited spontaneous contraction of the portal vein (Carruba, Mandelli & Mantegazza, 1973; Weston & Golenhofen, 1976). As the actions of vasopressin on membrane and mechanical properties in mesenteric vessels are poorly understood, the effects of this hormone on the membrane and contractile properties of large and small muscular arteries, and of large and small veins in the mesenteric system were investigated, and the findings were compared with the effects of noradrenaline (NA) and another neurohypophyseal hormone, oxytocin.

Methods

Guinea-pigs of either sex, weighing 300 to 350 g were stunned and bled. The cranial mesenteric artery (1 to 1.5 mm in diameter), jejunal mesenteric artery (0.2 to 0.4 mm in diameter), mesenteric vein (0.3 to 0.6 mm in diameter) and portal vein were excised and freed of connective tissue with the aid of a dissecting binocular microscope. Each tissue was mounted in an organ bath having a volume of 2 ml, without incising the vessel, except for the portal vein, and each end was pinned in position for studies of single cell activity. The perfusion was carried out at a rate of 3 ml/min and temperature was kept at 34 to 36°C. A glass microelectrode filled with 3 M KCl, having a resistance of more than 50 MΩ, was inserted into the cells from a serosal side of the vessels. In order to investigate the passive properties of the tissue, the partition stimulating method described by Abe & Tomita (1968) was used.

For isometric tension recording, the mesenteric artery and vein were cut in a helical direction to a width of about 1 mm in the jejunal mesenteric artery and mesenteric vein and 2 mm in the cranial mesenteric artery; a length of about 10 mm of these vessels was used. The portal vein was cut along the longitudinal axis 10 to 15 mm in length and 2 mm in width. The tissue was mounted in a vertical tubular bath with a volume of 2 ml. One end of the tissue was fixed at the bottom of the bath and the other end was connected to an isometric tension recorder by a thread. The flow rate of solution was 3 ml/min and temperature was fixed at 34°C.

Modified Krebs solution of the following ionic composition (mM) was used: Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134.0 and glucose, 11.5. The solution was aerated with 97% O_2 and 3% CO_2 and pH was adjusted to 7.2. In the excess $[K]_o$ solution, NaCl was replaced with KCl. In the Na-deficient solution, NaCl was replaced with an equimolar concentration of choline chloride with atropine 1 μg/ml or Tris chloride. For the reduction of Na which is contained in bicarbonate buffer to below 15.5 mM, $NaHCO_3$ was replaced with 5.9 mM $KHCO_3$ and 9.6 mM choline chloride or Tris chloride, 5.9 mM KCl being omitted.

The following drugs were used at concentrations described in the results; atropine sulphate (Tanabe) (±)-noradrenaline HCl (Sankyo), lysin-vasopressin (Sandoz), oxytocin (Sandoz), tetrodotoxin (Sankyo), tetraethylammonium chloride (TEA)- (Ishizu) ouabain (Merck), phentolamine mesylate (CIBA-Geigy Ltd.), propranolol (Sumitomo). The stock solution of vasopressin and oxytocin was 100 iu/ml and contained an acetate buffer and trichlorisobutyl-alcohol as a preservative. For each experiment, a fresh solution was prepared.

Results

Effects of vasopressin, oxytocin and noradrenaline on the membrane of jejunal mesenteric artery

Figure 1 shows effects of vasopressin, oxytocin and

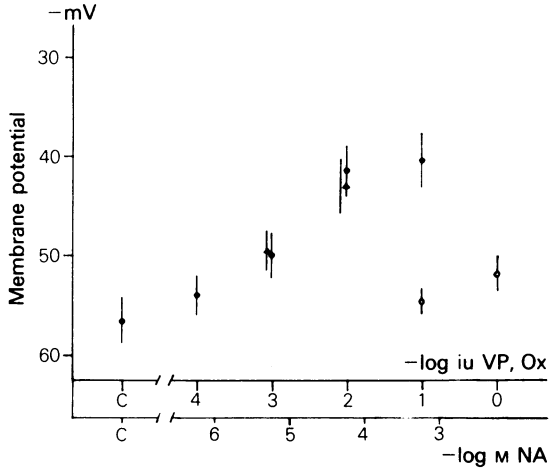

Figure 1 The effects of various concentrations of vasopressin (●, VP), oxytocin (○, Ox) and noradrenaline (▲, NA) on the membrane potential of jejunal mesenteric artery. Vertical bars indicate 2 × s.d.

NA on the membrane potential of jejunal mesenteric artery. As high concentrations of vasopressin or NA produced membrane activity, the membrane potentials were measured only when the membrane was depolarized by these substances before or in the absence of generation of membrane activities. The mean membrane potential [Em] was -56.6 ± 2.3 mV, s.d. ($n=120$). By application of various concentrations of vasopressin, oxytocin or NA, the membrane was depolarized dose-dependently. The minimum concentrations of vasopressin and oxytocin required to produce depolarization were 1×10^{-4} iu/ml (Em = -54.0 ± 2.0 mV, s.d., $n=25$) and 1 iu/ml (Em = 51.9 ± 1.8 mV, s.d., $n=25$), respectively. The depolarization induced by 5.9×10^{-5} M NA (Em = -42.8 ± 2.3 mV, s.d., $n=25$) showed much the same value as that induced by 1×10^{-2} iu/ml vasopressin (Em = -41.5 ± 2.5 mV, s.d., $n=25$).

To investigate the ionic mechanisms involved in the vasopressin-induced depolarization, the effects of vasopressin and NA on the membrane potential were observed in various ionic environments (Figure 2). Figure 2a shows the effect of vasopressin on the membrane potential in various concentrations of $[K]_o$; 1×10^{-2} iu/ml vasopressin and 5.9×10^{-5} M NA were used throughout the present experiment. In 59 mM $[K]_o$, the membrane was depolarized from -56.6 ± 2.3 mV, to -23.0 ± 1.5 mV, and vasopressin did not produce any further depolarization. In 17.7 mM $[K]_o$, membrane was depolarized to -41.5 ± 2.5 mV,

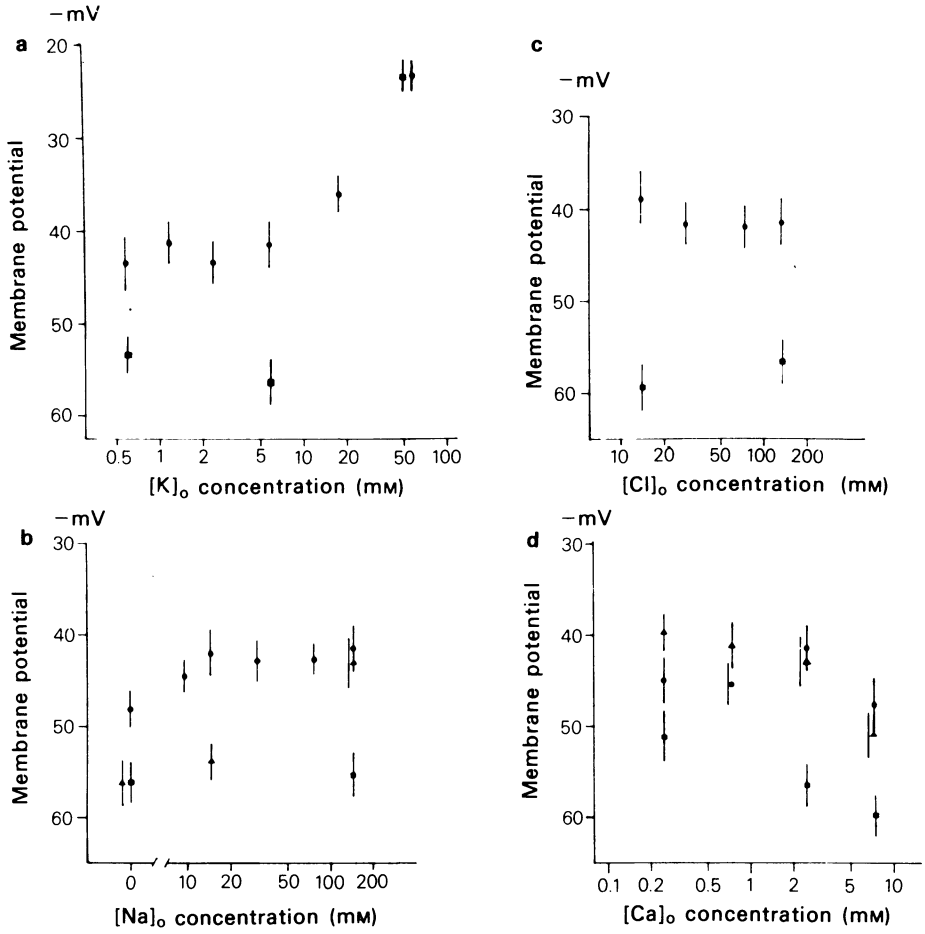

Figure 2 The effects of vasopressin (●, 1×10^{-2} iu/ml) and noradrenaline (NA) (▲, 5.9×10^{-5} M) on the membrane potential of jejunal mesenteric artery in various ionic environments; (■) = control. (a) The effects of various concentrations of $[K]_o$ on the membrane potential in the absence or presence of vasopression. (b) The effects of various $[Na]_o$ concentrations in presence or absence of vasopressin and NA on the membrane potential. (c) The effects of various $[Cl]_o$ on the membrane potential in the presence or absence of vasopression. (d) The effects of various $[Ca]_o$ on the membrane potential in the presence or absence of vasopression and NA. Vertical bars indicate 2 × s.d.

(n=25) and vasopressin further depolarized the membrane to −35.8 ± 2.1 mV, (n=15). When $[K]_o$ was reduced, the membrane was depolarized but the further depolarizations produced by vasopressin reached much the same value as those observed in Krebs solution (in 0.6 mM $[K]_o$ from −53.5 ± 2.1 mV, to −43.8 ± 3.1 mV, n=25).

Figure 2b shows the effects of vasopressin and NA on the membrane potential in Na-deficient and Na-free solutions in which $[Na]_o$ was replaced with choline chloride. A reduction in $[Na]_o$ slightly hyperpolarized the membrane. In a normal concentration of $[Na]_o$ solution, vasopressin (1×10^{-2} iu/ml) and NA (5.9×10^{-5} M) showed much the same depolarization. In 15.4 mM $[Na]_o$, the vasopressin-induced depolarization was slight (−41.5 ± 2.5 mV, in control solution to −42.0 ± 3.1 mV, n=20 in 15.4 mM $[Na]_o$) but the NA-induced depolarization was markedly suppressed (−42.8 ± 2.3 mV, in control solution to −53.8 ± 2.1 mV, n=20 in 15.4 mM $[Na]_o$). In Na-free solution, NA-induced depolarization was completely suppressed (−56.1 ± 2.6 mV, n=20), while the vasopressin-induced depolarization was still apparent (−46.5 ± 2.1 mV, n=20). This phenomenon was also consistently observed in Na-deficient solutions in which $[Na]_o$ was replaced with TRIS.

When $[Cl]_o$ was replaced with propionate, the membrane was slightly hyperpolarized (−56.6 to −59.7 ± 2.5 mV, n=20). Figure 2c shows the effects of vasopressin (1×10^{-2} iu/ml) on the membrane potential in various concentrations of $[Cl]_o$. The depolarization of the membrane produced by VP was slightly increased in 15.4 mM $[Cl]_o$ from −59.7 ± 2.5 mV, to −38.8 ± 2.7 mV., n=20).

A reduction or an increase in $[Ca]_o$, respectively caused the membrane to be either depolarized or hyperpolarized (Figure 2d). In 7.5 mM $[Ca]_o$, the membrane was hyperpolarized (from −56.6 ± 2.3 mV, to −59.8 ± 2.0 mV, n=25). The NA-induced depolarization was smaller than the vasopressin-induced depolarization (NA; −51.8 ± 2.8 mV, n=15, vasopressin; −47.7 ± 2.8 mV, n=15). In low concentrations of $[Ca]_o$, the membrane was depolarized (to −51.5 ± 2.8 mV, n=25 in 0.25 mM $[Ca]_o$, −56.6 ± 2.3 mV, n=25 in 0.75 mM $[Ca]_o$), and NA-induced depolarization was consistently preserved, however,

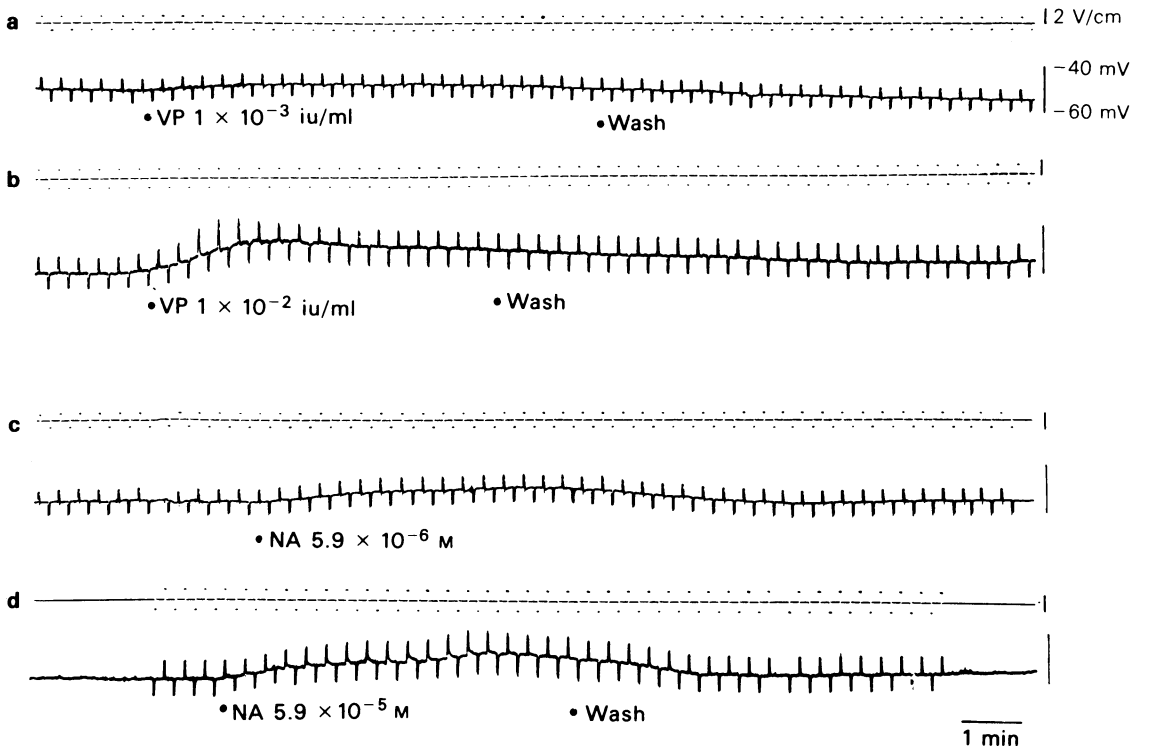

Figure 3 The effects of vasopressin (VP) and noradrenaline (NA) on the membrane potential and electrotonic potential (jejunal mesenteric artery). Stimulus duration is 1.5 s. (a) application of vasopressin (VP) 1×10^{-3} iu/ml; (b) application of vasopressin (VP) 1×10^{-2} iu/ml; (c) application of noradrenaline (NA) 5.9×10^{-6} M; (d) application of noradrenaline (NA) 5.9×10^{-5} M. (●) Application and washout of the drug.

vasopressin-induced depolarization was suppressed (vasopressin: -45.0 ± 2.5 mV, $n=15$, NA: -39.8 ± 2.1 mV, $n=15$ in 0.75 mM $[Ca]_o$).

To clarify the nature of the depolarization, inward and outward current pulses (1.5 ms duration) were alternately applied before, during and after treatment with vasopressin or NA (Figure 3). The microelectrode was inserted into the same single cell at a distance of about 0.1 mm from the stimulating electrode. In this preparation, outward current pulses generated the graded responses. Vasopressin (1×10^{-3} iu/ml and 1×10^{-2} iu/ml) and NA (5.9×10^{-6} M and 5.9×10^{-5} M) depolarized the membrane and increased the amplitude of the electronic potential evoked by inward current pulses. The evoked graded responses were increased in amplitude during the depolarization of the membrane, in the presence of these agents.

To investigate further the effects of vasopressin and NA on the membrane resistance, the current-voltage relationship was observed before and during application of vasopressin or NA (Figure 4). A microelectrode was inserted into cells at distances of 0.1 mm (for vasopressin) and 0.15 mm (for NA) from the stimulating electrode. Vasopressin (1×10^{-3} iu/ml) and NA (5.9×10^{-6} M) depolarized the membrane. The current-voltage relationship measured by application of inward current pulses was consistently steeper during application of vasopressin or NA than that observed in the control. Vasopressin or NA raised the potential required for generation of the rectifying property of the membrane produced by outward current pulses. However, depolarization of the membrane induced by different procedures such as high concentrations of $[K]_o$ did not produce an increase in the membrane resistance, despite depolarization. Therefore, an increase in the membrane resistance in the presence of vasopressin or NA is due not to a passive change of the membrane but rather to a decrease in the ionic permeability of the membrane.

When vasopressin (1×10^{-2} iu/ml) was applied repetitively in Krebs solution, the resting membrane was hyperpolarized to -65 mV after washing with Krebs solution for 30 to 60 min. Even if the membrane was hyperpolarized about 10 mV, the depolarization induced by vasopressin reached the same potential level as that observed in the first trial. These changes in the membrane potential were not observed after treatment with NA. To clarify whether this hyperpolarization induced by repetitive treatment with vasopressin is due to an activation of the electrogenic Na pump or due to increase in K permeability, the effects of ouabain were observed. The depolarization of the membrane induced by ouabain (1.3×10^{-6} M) was about 7 mV in both normal membrane potential and increased membrane potential, after repetitive application of vasopressin. From this observation, the increase in membrane

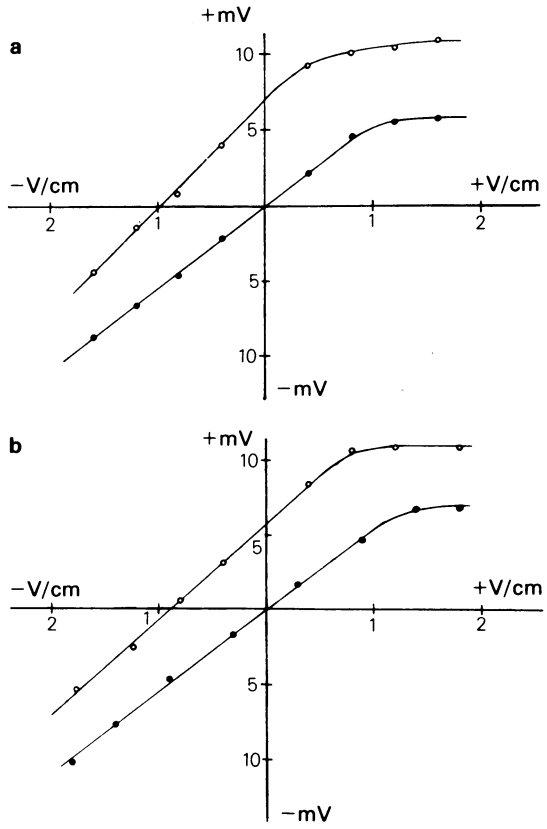

Figure 4 The effects of vasopressin (VP) (1×10^{-3} iu/ml) and noradrenaline (NA) (5.9×10^{-6} M) on the current-voltage relationship of the jejunal mesenteric artery. (a) Before and during application of vasopressin (●); (○) = control. (b) Before and during application of noradrenaline (NA) (●); (○) = control.

potential is thought due to an increase in the K-conductance.

Effects of vasopressin and noradrenaline on phasic membrane activities of jejunal mesenteric artery

Phasic membrane activities of smooth muscle cells of the jejunal mesenteric artery were induced by vasopressin or NA in normal and various ionic environments. Figure 5 shows the membrane activities induced by vasopressin in normal Krebs solution. At a concentration of 1×10^{-4} iu/ml, vasopressin produced a depolarization of the membrane (a_1) and at 1×10^{-3} iu/ml, it depolarized the membrane and sometimes produced a small slow wave but did not generate spikes (a_2). With further increase in the concentration (1×10^{-2} iu/ml), vasopressin produced various membrane activities of different durations. When the membrane was depolarized, a slow wave gradually developed, increasing in amplitude, and

after 2 to 3 min, the magnitude of the slow wave reached a maximum then declined more gradually to cease in 10 to 20 min, even in the continued presence of vasopressin (a_3). The amplitude and frequency of these slow waves varied from cell to cell (amplitude: 5 to 40 mV; frequency: 2 to 8/min). When the slow wave was large, the peak of the repolarization phase sometimes exceeded the resting membrane potential before application of vasopressin (a_3). When the amplitude of the slow waves enlarged the waves often fused, resulting in an apparent decrease in wave frequency. In general, when the membrane potential was high, vasopressin depolarized the membrane to a greater extent, enlarged the amplitude and reduced the frequency of the slow wave. Figure 5 shows spike generation on a depolarizing phase with subsequent spike and slow wave complex. Usually the first spike occurred from a high membrane potential as a single shape and independent of the following membrane activity. When the membrane was maximally depolarized, a slow wave began to develop with increasing amplitude. In this cell, several spikes were generated on the top of the slow wave. This may be called 'spikes on slow wave type'. The relationship between the slow waves and the spikes varied. When the slow wave was large, several spikes appeared with a small amplitude. When the slow wave was small, a single large spike was superimposed on the slow wave. Figure 5 c shows a train of spikes evoked by vasopressin. In this type, the slow wave was not clearly observed and may be called 'spike type'; its appearance was rare. In most cases, membrane activity appeared as slow wave type or spikes on the slow wave type. Figure 5 d_1 shows a typical pattern of spikes on a slow wave type produced by vasopressin. A spike evoked by rapid depolarization of the membrane was followed by a stable phase after which a gradually increasing slow wave occurred. Several small spikes merged to become one on top of the slow wave, then slow waves without spikes followed as the amplitude of the slow wave gradually decreased. As shown in Figure 5 d_2, when vasopressin was washed

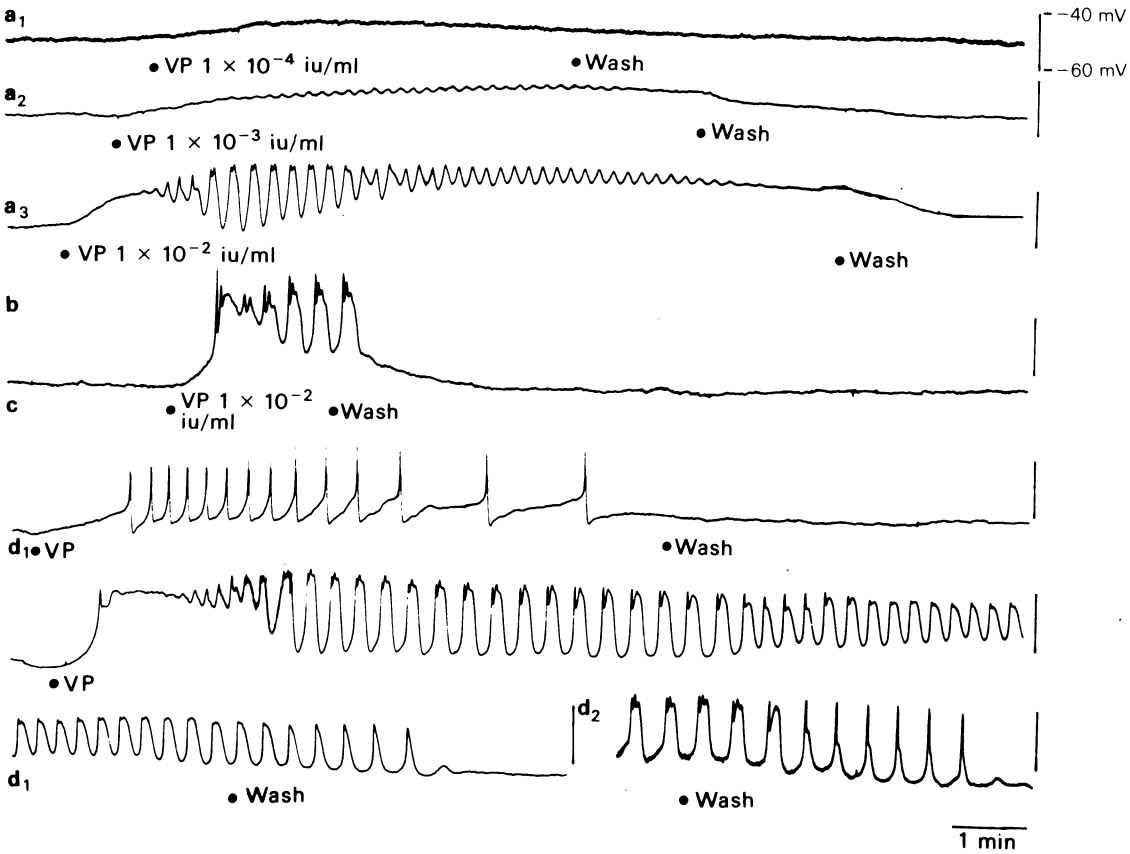

Figure 5 Membrane activities of jejunal mesenteric artery by application of various concentrations of vasopressin (VP). (a_1), (a_2) and (a_3), (d_1) and (d_2) were recorded from the same muscle preparation. (a_1) 1×10^{-4} iu/ml vasopressin; (a_2) 1×10^{-3} iu/ml vasopressin; (a_3), (b), (c), (d_1), (d_2) 1×10^{-2} iu/ml vasopressin. (●) Application and washout of vasopressin; (d_2) washout at the maximal amplitude of the slow wave.

out at the maximum amplitude of the slow waves, the dissociation of each component of the slow wave and spike was observed during the recovery process. These phenomena indicate that generation of the slow wave and spike induced by vasopressin differs in nature.

By prolonged treatment with vasopressin, membrane activity disappeared, the membrane gradually repolarized and in 10 to 20 min completely repolarized to the control potential.

Membrane activities induced by vasopressin were observed in various concentrations of $[K]_o$. In excess $[K]_o$ (59 mM or 17.7 mM), vasopressin did not produce any membrane activity. With a reduction in $[K]_o$, the membrane was depolarized and the amplitude of the depolarization induced by vasopressin was small. Slow waves in low concentrations of $[K]_o$ had a low amplitude with a high frequency. This was compatible with the observation in Krebs solution that the amplitude of the slow wave was dependent on the membrane potential.

Membrane activities induced by vasopressin varied with concentrations of $[Na]_o$ (Figure 6). In 15.4 mM $[Na]_o$, the activity of the membrane and amplitude of the depolarization to vasopressin were much the same as those observed in the control solution. However, in 9.5 mM $[Na]_o$, the depolarization of the membrane and its activity were suppressed, and in Na-free solution, membrane activity was completely suppressed, though a small depolarization was still apparent. In low concentrations of $[Cl]_o$, membrane activities were much the same as those observed in the control solution.

Membrane activity induced by vasopressin was investigated in various concentrations of $[Ca]_o$. In 7.5 mM $[Ca]_o$, the membrane was hyperpolarized and the amplitude of the depolarization induced by vasopressin was much the same as the control but membrane activity was suppressed. In contrast, in low concentrations of $[Ca]_o$ the membrane was depolarized, and vasopressin-induced depolarization was smaller than the control. As a consequence, the amplitude of the slow wave was slightly decreased, but the slow waves were not suppressed. However, spikes on the slow waves were suppressed, so that only the slow wave type was observed.

The NA-induced slow wave was smaller in amplitude and shorter in duration than the vasopressin-

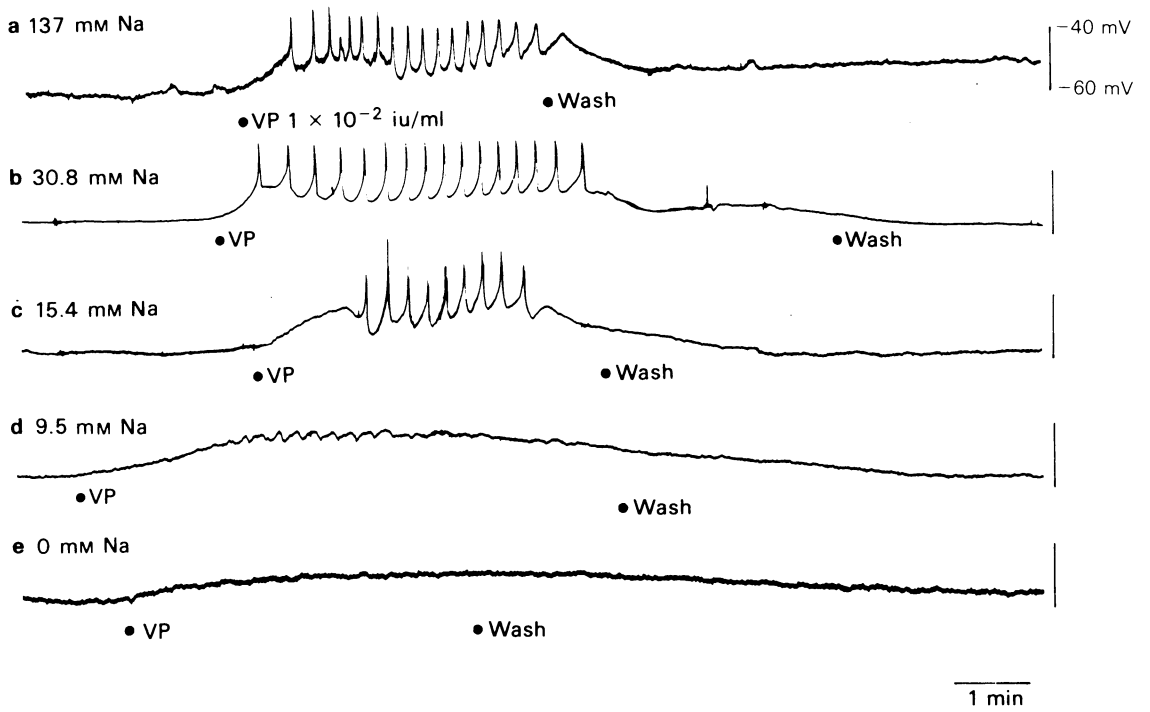

Figure 6 Membrane activities of smooth muscles cells of jejunal mesenteric artery by application of vasopressin (1×10^{-2} iu/ml) in various concentrations of $[Na]_o$.
A–E, all records were taken from the same preparation. Symbols in the figure are the same as those described in Figure 5.

induced slow wave, though the depolarization induced by both substances was much the same. The desensitization by prolonged treatment with NA was evident for a longer period than the desensitization seen with vasopressin. After vasopressin-induced membrane activities disappeared due to desensitization, membrane activities was re-induced by NA, and *vice versa*. This indicates that disappearance of membrane activity is not due to inactivation of the slow wave, but rather to desensitization by each drug. In various concentrations of $[Ca]_o$, membrane activity produced by NA was similar to that induced by vasopressin. However, in 15.4 mM $[Na]_o$, membrane activity was not produced by NA.

Figure 7 a, b and c show the effects of various drugs on membrane activity induced by vasopressin (1×10^{-2} iu/ml) or NA (5.9×10^{-5} M). Tetraethylammonium (TEA 5 mM) with vasopressin or NA produced membrane activity in the preparation not generated by vasopressin or NA alone (a_1 and a_2). TEA produced large spikes on a depolarizing phase. These spikes had a higher frequency (10 to 11/min) and shorter duration (1 to 3 min) than slow waves. Application of 10 mM TEA without addition of vasopressin or NA depolarized the membrane about 5 mV, and sometimes produced spontaneous spikes but did not produce a slow wave. Membrane activities induced by vasopressin or NA were not affected (Figure 7 b and c) by pretreatment with ouabain (1.3×10^{-6} M) and tetrodotoxin (3.1×167 M). Furthermore, the membrane activity induced by vasopressin was not affected by phentolamine (3.9×10^{-7} M) or propranolol (3.6×10^{-7} M). These observations indicate that the membrane activities induced by vasopressin or NA are due to direct actions on the muscle membrane. The membrane potential, the amplitude of depolarization and slow wave generations produced by vasopressin were not affected (Figure 7 d) in the presence or absence of $[Mg]_o$.

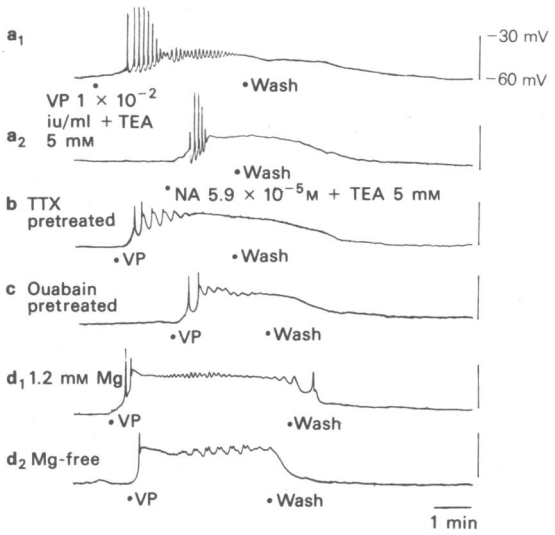

Figure 7 Effects of drugs or ions on membrane activity of jejunal mesenteric artery by application of vasopressin (VP) (1×10^{-2} iu/ml) or noradrenaline (NA) (5.9×10^{-5} M). (a_1) and (a_2) 5 mM TEA was applied at the same time as vasopressin (VP) or noradrenaline (NA), respectively; (b) pretreated with tetrodotoxin (TTX) (3.1×10^{-7} M); (c) pretreated with ouabain (1.3×10^{-6} M); (d_1) control; (d_2) effects of Mg-free solution on the membrane activities induced by vasopressin.

Oxytocin (1 iu/ml) depolarized the muscle membrane of jejunal mesenteric artery without generation of electrical activity.

Effects of vasopressin, noradrenaline and oxytocin on membrane properties of cranial mesenteric artery and mesenteric and portal veins

In the cranial mesenteric artery, whilst 5.9×10^{-7} M NA depolarized the membrane about 3 to 5 mV, vasopressin (1 iu/ml) and oxytocin (1 iu/ml) produced neither excitatory nor inhibitory actions.

In the mesenteric vein anatomically located in a parallel position to the jejunal mesenteric artery, NA depolarized the membrane (4.3 ± 1.5 mV, $n=15$), but vasopressin (1 iu/ml) and oxytocin (1 iu/ml) did not produce any effect. In the portal vein, NA (5.9×10^{-8} M) produced a depolarization of about 3 mV and increased spontaneous electrical activity, but oxytocin and vasopressin (both 1 iu/ml) produced no change in the membrane potential.

Effects of vasopressin, oxytocin and noradrenaline on mechanical responses in cranial and jejunal mesenteric arteries and mesenteric and portal veins

In the jejunal mesenteric artery (Figure 8 A), vasopressin produced a dose-dependent contraction. The vasopressin-induced contraction (1×10^{-2} iu/ml) was larger than the 127 mM $[K]_o$-induced contraction, but the NA-induced contraction (5.9×10^{-5} M) was smaller than 127 mM $[K]_o$-induced contraction, though the vasopressin-induced depolarization was much the same as the NA-induced one. In 0.25 mM $[Ca]_o$, the contraction induced by vasopressin or NA was suppressed (Figure 8 A II). In 15.4 mM $[Na]_o$, the vasopressin-induced contraction was slightly suppressed, while the NA-induced contraction was markedly suppressed. In NA-free solution, the vasopressin-induced contraction was completely suppressed (Figure 8 A III). In K-free solution, contractions produced by vasopressin (1×10^{-2} iu/ml) and NA (5.9×10^{-5} M) (Figure 8 A IV) were similar to those in the control solution.

In the cranial mesenteric artery (Figure 8 B), NA 5.9×10^{-6} M or 5.9×10^{-5} M produced a marked contraction, whereas, vasopressin $1 \times$ iu/ml or oxytocin $1 \times$ iu/ml did not. In 0.25 mM $>Ca]_o$, the contraction induced by NA in the cranial mesenteric artery was preserved to a greater extent than that in jejunal mesenteric artery. In K-free solution, NA-induced contraction was larger than that in control solution. In the mesenteric vein (Figure 8 C), NA (5.9×10^{-5} M) produced a small contraction compared with the 127 mM $[K]_o$-induced contraction whilst in the portal vein (Figure 8 D), NA (5.9×10^{-8} to $5.9 \times$

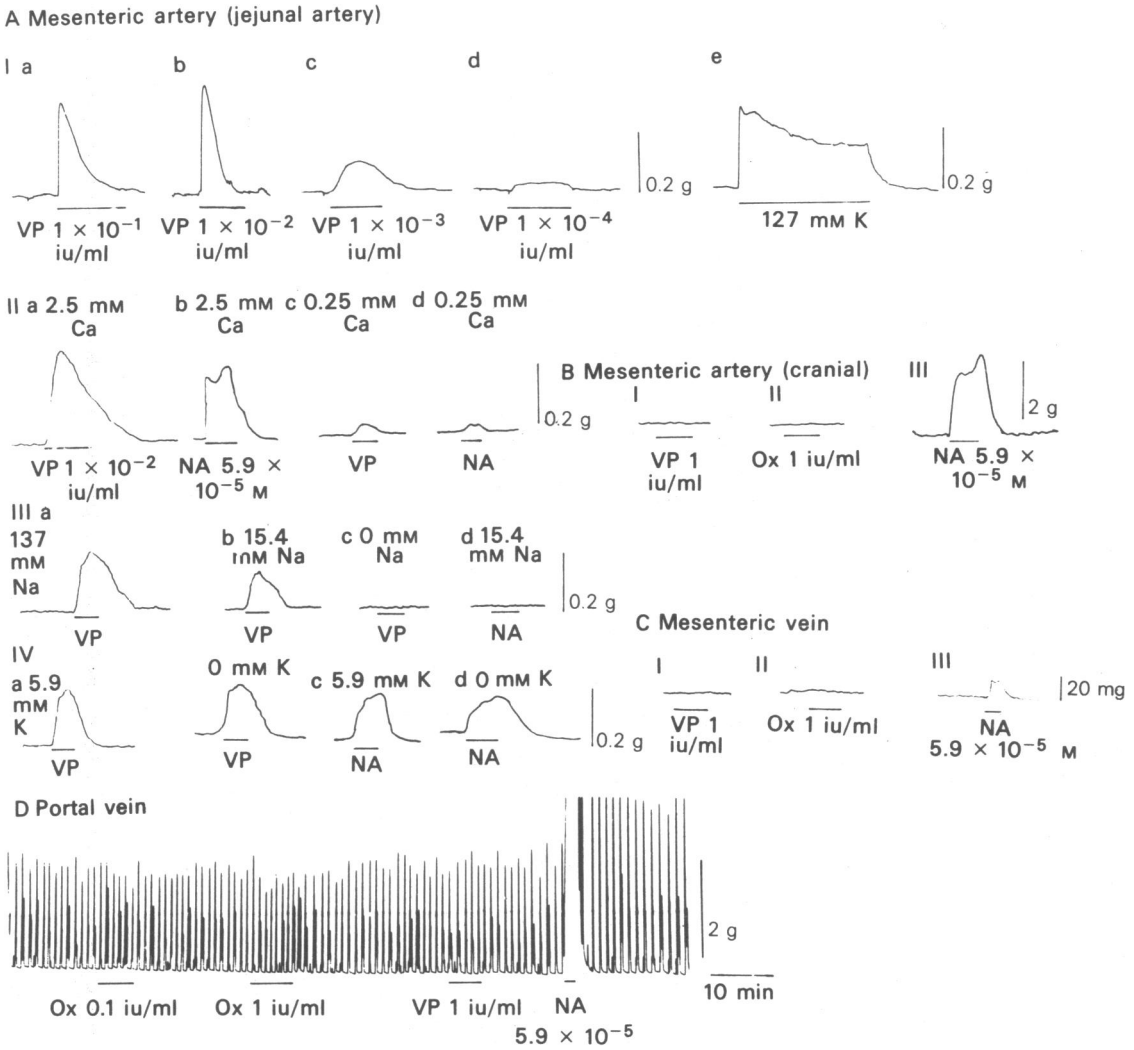

Figure 8 The mechanical responses of some mesenteric vessels to vasopressin (VP), oxytocin (Ox) and noradrenaline (NA). (A) Jejunal mesenteric artery: (I) Responses to various concentrations of vasopressin. (II) Responses to vasopressin (1×10^{-2} iu/ml) and noradrenaline (5.9×10^{-5} M) in a low concentration of $[Ca]_o$. (a) and (b) control; (c) and (d) in 0.25 mM $[Ca]_o$. (III) Responses to vasopressin (1×10^{-2} iu/ml) and noradrenaline (5.9×10^{-5} M) in low $[Na]_o$. (a) control; (b) and (c) response to vasopressin in $[Na]_o$ 15.4 mM or $[Na]_o$-free. (d) response to noradrenaline in $[Na]_o$ 15.4 mM. (IV) Vasopressin (1×10^{-2} iu/ml) and noradrenaline (5.9×10^{-5} M) in low $[K]_o$. (a) and (c) control, (b) and (d) $[K]_o$-free. (B) Responses of cranial mesenteric artery to vasopressin (VP), oxytocin (Ox) and noradrenaline (NA). (C) Responses of mesenteric vein to vasopressin (VP), oxytocin (Ox) and noradrenaline (NA). (D) Responses of portal vein to vasopressin (VP), oxytocin (Ox) and noradrenaline (NA). Horizontal bars indicate application of each drug.

10^{-6} M) produced a large contraction. Vasopressin and oxytocin produced neither excitatory nor inhibitory effects in either mesenteric or portal veins.

Discussion

Steedman (1966) studied the smooth muscle cell activity of the rat mesenteric artery *in vivo*, using a microelectrode or a pressure extracellular method and reported that rhythmic slow waves varying from 4.5 to 7.8 s and spontaneous spikes occurred. In addition locally applied NA (10 ng to 1 μg/ml) and vasopressin (2.5×10^{-4} iu/ml) caused a marked increase in firing of action potentials without any marked change in the duration of slow waves. *In vitro* experiments in the guinea-pig, showed that spontaneous action potentials and slow waves were not observed, though TEA, Ba or nerve stimulation evoked spike generation (Harder & Sperelakis, 1978; Suzuki & Kuriyama, personal communication).

In a large elastic artery, the membrane is electrically quiescent. When NA or high $[K]_o$ are applied, a slow wave is produced in the sheep carotid artery and in the rabbit aorta (Keatinge, 1964; 1978; Mekata, 1979). The latter author concluded that since the slow wave was suppressed with administration of phentolamine, high K-induced slow potentials were not evoked by the high concentration of $[K]_o$ directly but were evoked by NA released by the high concentration of $[K]_o$. As the increase in membrane conductance in the slow wave produced by NA was much higher than that produced by excess $[K]_o$, Mekata (1979) suggested that the generation of slow potential was presumably due to an increase in the Na-conductance.

Some differences in membrane properties recorded from the rabbit aorta and jejunal mesenteric artery of the guinea-pig were apparent, e.g. (i) in the jejunal mesenteric artery, the slow wave and spike complex were observed with application of vasopressin or NA. However, spikes on the slow wave were not recorded in the aorta; (ii) the amplitude and frequency of slow waves of the mesenteric artery were more variable than those in the rabbit aorta (mean frequency, 2.6 cycles/min, range 1.1 to 4.2; mean amplitude 18.6 mV, range 16 to 26 in aorta) (Mekata, 1979); (iii) the membrane resistance of the mesenteric artery increased during the depolarization induced by both vasopressin and NA but that of the aorta decreased during the depolarization induced by NA; (iv) high $[K]_o$ solution never produced spikes and slow waves in the guinea-pig jejunal mesenteric artery, but produced slow waves in the rabbit aorta.

High $[K]_o$ solutions depolarized the membrane and reduced the membrane resistance; the passive depolarization of the membrane induced by outward current pulses also produced rectifying properties in the jejunal mesenteric artery. Therefore, an increase in the membrane resistance induced by NA or vasopressin was not due to a depolarization, but rather to an active response of the membrane. An increase in the membrane resistance during application of NA has been reported in the case of smooth muscles of the guinea-pig ureter, and the depolarization induced by NA is thought to be mainly due to a decrease in the K permeability (Shuba, 1977). In this experiment, the depolarization might also be explained by a reduction in the K-conductance.

Current-voltage relationships showed that outgoing rectification shifted to a more depolarized level, probably due to a reduction in the K-conductance. It has been reported that TEA depolarized the membrane, increased the membrane resistance, suppressed the rectifying property and generated the graded response by outward current in the guinea-pig pulmonary artery (Casteels, Kitamura, Kuriyama & Suzuki, 1977). Furthermore, in this tissue, TEA added to the spike generation of vasopressin or NA but although TEA alone produced spontaneous spike generation, it did not activate the slow wave. Therefore, the suppression of rectifying properties induced by vasopressin or NA may contribute to the generation of spikes and TEA-induced suppression of rectifying properties may also act on the spike generation resulting from application of vasopressin or NA. In low concentrations of $[Ca]_o$, spikes produced by both vasopressin and NA were suppressed, though slow waves were still generated. In low concentrations of $[Na]_o$ (down to 13.4 mM) spikes were not inhibited. Thus it is suggested that the spikes which appeared on the slow waves may be due to activation of the Ca channel, as suggested by Harder & Sperelakis (1979) to explain the spike generation on addition of TEA.

The ionic mechanism of the slow wave generation seems to be more complicated. In a low concentration of $[K]_o$, $[Na]_o$, $[Ca]_o$, or $[Cl]_o$, the vasopressin-induced slow wave was fairly well preserved, as long as there was an adequate depolarization. When the depolarization of the membrane was reduced by the further reduction in $[Na]_o$ to zero, the slow wave was inhibited. Excess $[Ca]_0$ suppressed the slow wave induced by vasopressin, but did not suppress the depolarization. These phenomena suggest that an increase in the Na-conductance contributes to generation of the slow wave.

The NA-induced depolarization was suppressed in low concentrations of $[Na]_o$ and a high concentration of $[Ca]_o$, while vasopressin-induced depolarization was less affected in these solutions. Osa & Taga (1973) reported that in the rat myometrium, both carbachol and oxytocin depolarized the membrane and increased the conductance of the membrane, and the depolarization produced by carbachol was

reduced in solutions containing a low concentration of $[Na]_o$ or excess $[Ca]_o$, while the depolarization produced by oxytocin was little affected in any of the above ionic solutions. They, therefore, speculated that the depolarization induced by carbachol or oxytocin differed in nature.

In general, vasopressin and oxytocin are thought to act on the same type of smooth muscle receptors, such as in the myometrium and blood vessels, though the affinity differs with tissue (Somlyo & Somlyo, 1970). In the present experiments, vasopressin and oxytocin acted on the same regions in mesenteric vessels, though vasopressin showed a 1000 times greater potency than oxytocin in activating the membrane. In the mesenteric artery, both vasopressin and NA depolarized the membrane, mainly due to a reduction in the K-conductance, however, contributions of an increase in the Na-conductance and Ca-conductance differed with regard to generation of the depolarization. In addition, the desensitization induced by prolonged treatment with either vasopressin or NA did not affect the actions of successively applied NA or vasopressin, and vasopressin actions were not inhibited by adrenoceptor blocking agents. From these observations it is postulated that vasopressin and NA act on different receptors and thereby have a different effect on ionic channels.

Mg ions potentiate the contractile actions of neurohypophyseal hormones on all types of smooth muscle so far investigated (Altura, 1974). However, in 1.2 mM $[Mg]_o$ or Mg-free solution, no difference in depolarization and membrane activity was observed. This observation hardly supports the postulate that Mg ions primarily affect the affinity or binding of these hormones to their receptor.

Takata (1980) observed that in the mesenteric system, responses of the membrane to various concentrations of $[K]_o$, acetylcholine and phenylephrine differed between jejunal and cranial mesenteric arteries, and also between mesenteric and portal veins. In the experiments described here, responses of the muscle membrane to NA, vasopressin and oxytocin also differed with region. In the portal vein, an inhibitory response induced by vasopressin or oxytocin was reported by Carruba *et al.* (1973) and Weston & Golenhofen (1976) but such an inhibition was not observed in the present experiments. This discrepancy is probably due to differences in the concentrations of chlorobutanol added as a preservative. In fact, when we used oxytocin (5 iu/ml with 5 mg chlorobutanol as stock solution, Teikoku Zoki Co.) in a concentration of 5×10^{-2} iu/ml, the membrane of the portal vein was hyperpolarized and spike activity was inhibited.

From these observations, it is concluded that vasopressin acts on only small muscular arteries which control blood pressure, while NA acts on all mesenteric vessels, and that the portal vein is more sensitive than mesenteric arteries to NA; thus, vasopressin probably reduces and NA increases hepatic portal vascular resistance.

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