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MECHANISM OF ACTION OF ANGIOTENSIN II ON EXCITATION-CONTRACTION COUPLING IN THE RAT PORTAL VEIN

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1 The action of angiotensin II (At II) has been studied on the electrical and mechanical activity of the vascular smooth muscle of the rat portal vein.

2 At low concentrations (between 5×10^{-10} and 10^{-9} M) At II induces an acceleration of spontaneous action potential (AP) discharge without change in the resting membrane potential. The frequency and size of the associated contractions are simultaneously augmented. Under these conditions the size of the spikes is not affected, thus suggesting that At II triggers the release of Ca^{2+} from internal stores.

3 The increase in AP discharge rate produced by low concentrations of At II results from an acceleration of the pacemaker potential. Furthermore, in the presence of 10 mM tetraethylammonium (TEA), there is an acceleration of the repolarizing phase of AP.

4 Ouabain (10^{-3} M) inhibits the increase in rhythmic activity induced by low concentrations of At II (in the presence of 10 mM TEA), thus suggesting that the Na-K pump is directly or indirectly involved in this action of the peptide.

5 At higher concentrations, At II produces a concentration-dependent depolarization with an EC_{50} of 1.2×10^{-8} M and a maximum of 10^{-7} M. The associated contraction has an EC_{50} of 3.3×10^{-8} M and a maximum of 3×10^{-7} M.

6 Ouabain (3×10^{-3} M) depolarizes the cell membrane. Under these conditions, At II (10^{-7} M) has a slight depolarizing effect, but it still produces a large tonic contraction.

7 It is concluded, that At II acts on different steps of excitation-contraction coupling, depending on the concentration. At low levels, the peptide mainly accelerates spike discharge, through a mechanism involving the Na-K pump. At higher concentrations, At II depolarizes the cell membrane. The contraction is then activated by the influx of Ca^{2+} due to secondary AP discharge and the release of Ca^{2+} from intracellular stores. Pharmacomechanical coupling has an important role in the triggering of contractions both at high and at low concentrations of At II.

Introduction

In addition to its potent vasoconstrictor activity, the octapeptide angiotensin II (At II) is also able to contract visceral smooth muscles such as guinea-pig taenia-coli and rat uterus (Regoli, Rioux & Park, 1974). We have shown previously (Hamon & Worcel, 1979) that the stimulating effect of At II on the rat uterus is associated with a depolarization due to an increase in Na^+ and K^+ conductances. We have also shown in the same study that the tonic component of contraction, i.e. the component which remains after the suppression of action potentials (APs), is partly triggered by the release of intracellular or membrane bound calcium. Concerning the action on vascular smooth muscles, At II has been reported to depolarize large peripheral arteries without spike production (Somlyo & Somlyo, 1970) whereas an increase in spike frequency associated with an increase in perfusion pressure has been observed in rabbit cerebral arteries (Lusamvuku, Sercombe, Aubineau & Seylaz, 1979). We have chosen the rat portal vein as an experimental model because of its great sensitivity to At II (Bohr & Uchida, 1967; Carruba, Mandelli & Mantegazza, 1973). Furthermore, similarities between this vessel and arterial microvessels have been observed (Ljung, 1970; Grände, 1979). At II induces an increase in AP frequency and a depolarization associated with an increase in contractility in the rat and rabbit portal veins whereas it produces a decrease in electrical and mechanical activity in the guinea-pig portal vein (Weston & Golenhofen, 1976). The purpose of the present study was to investigate further the mode of action of At II on the rat portal vein and more precisely, the mechanism responsible for the acceler-

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ation of AP frequency at low concentrations of the peptide, which appear to be more physiologically relevant (Powell-Jackson & MacGregor, 1976).

Methods

Male Wistar rats weighing 180–200 g were killed by a blow on the head and the portal vein was carefully removed. The vessel was placed in a dissecting dish containing a modified Krebs solution (see composition below) at room temperature. The connective tissue and part of the longitudinal muscle was carefully removed; then a short ring of 0.5–0.8 mm width was cut, and mounted according to a method described by Mulvany & Halpern (1977), in order to record electrical and mechanical activity simultaneously. Briefly, two tungsten wires (diameter 25 μm) were introduced into the lumen of the ring and then secured by the mean of stainless-steel screws into two Plexiglass holders as shown in Figure 1. One of the holders was fixed on the lever of a force transducer (BG 5, Kulite International) and the other one was attached to a hydraulic micromanipulator (HMD-1M Hydraulic microdrive, Clark Electromedical Instruments). This set up was placed in a thermostatically controlled perfusion chamber with a volume of 1.8 ml at 37°C unless otherwise stated. The electrical recordings were performed with conventional glass microelectrodes with a tip diameter of less than 0.5 μm, filled with KCl 3M and having a resistance of 30 to 60 MΩ and a tip potential of less than 5 mV. The electrodes were connected to a M707 WPI microprobe amplifier with capacitance compensation circuit. The maximum rate of rise of AP was measured with a differentiator having an RC constant of 1 ms. The criteria of impalements were those described by Kao & Nishiyama (1964). Drug-induced depolarization was sometimes compensated for by applying a hyperpolarizing constant current through large external silver electrodes, one of which was in contact with the preparation and the other was placed at the bottom of the chamber. This procedure was sometimes necessary in order to be able to compare the amplitude of action potentials (APs) in control and in At II containing solutions, at a similar membrane potential.

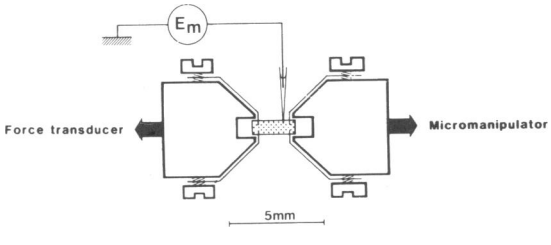

Figure 1 Diagram of the recording set-up showing the preparation (stippled rectangle) between the two Plexiglass holders. Two tungsten wires are introduced into the lumen of the ring of portal vein and secured on the two holders.

With this set up, impalements could be maintained for up to 2 h in the same cell with continuous mechanical and electrical recording.

The modified Krebs solution (Bülbring, 1953) used for dissection and perfusion in the chamber had the following composition (mM): NaCl 120.8, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.5, NaH_2PO_4 1.2 and glucose 11.5. It was bubbled in the reservoir as well as in the perfusion chamber with a 95% O_2/5% CO_2 gas mixture. The pH of the solution was 7.2. When manganese ions (Mn^{2+}) were used to block calcium channels, a HEPES buffered solution with the following composition (mM) was used throughout the experiment: NaCl 120.8, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, HEPES (N-2-hydroxyethylpiperazine-N'2- ethanesulphonic acid) 5.8, glucose 11.5. The pH of this solution was adjusted with NaOH to 7.3. In this case pure oxygen was used to bubble the perfusing solution. When tetraethylammonium chloride was used, it was added in place of an equimolar amount of sodium chloride.

The following drugs were used: HEPES (Calbiochem), tetrodotoxin (Sigma), phentolamine methane sulphonate (Ciba), tetraethylammonium chloride (TEA) (BDH), ouabain (Sigma), Asn^1, Val^5-angiotensin II (Hypertensin, Ciba). All the other salts were reagent grade and all solutions were made with double distilled water. Drugs were added to the perfusing solutions. The perfusion was always adjusted to a rate of 3 ml/min. The concentrations indicated in the text are final concentrations in the perfusing chamber.

Results

Spontaneous electrical activity of the portal vein

As described by many authors (Funaki & Bohr, 1964; Cuthbert & Sutter, 1964; Axelsson, Wahlström, Johansson & Jonsson, 1967; Hermsmeyer, 1971) the rat portal vein showed spontaneous electrical activity which was quite well correlated with mechanical activity in our experimental conditions. The spontaneous APs had an amplitude of 20 to 50 mV; overshoots were rarely observed. The resting membrane potential measured at the most negative point between bursts of APs was -52.5 ± 0.6 mV (47 cells, 30 preparations); the maximum and minimum values were -42 mV and -65 mV.

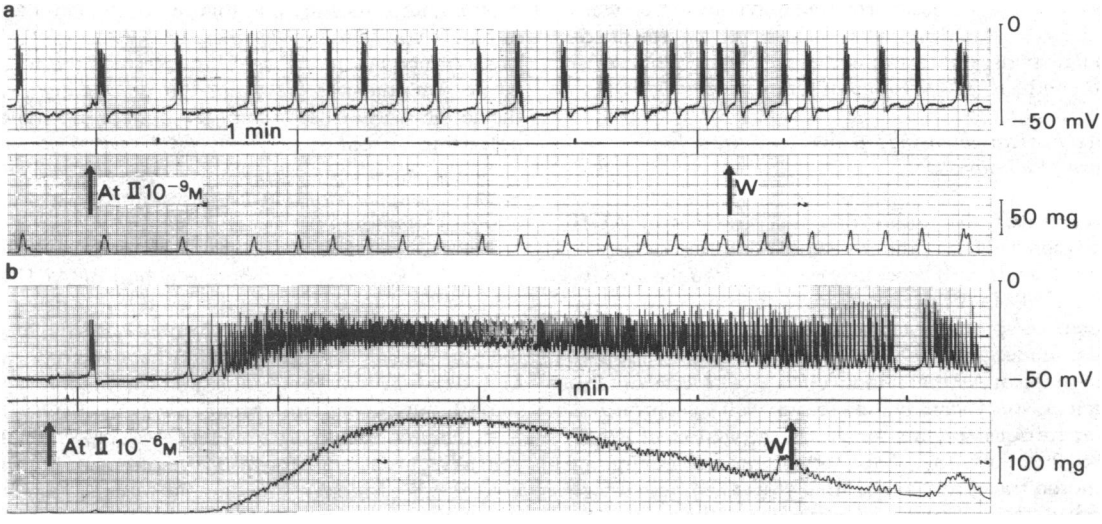

Figure 2 Action of angiotensin II (At II) on the spontaneous activity of rat portal vein. (a) At II 10^{-9} M accelerates the bursts of action potentials (APs) and associated phasic contractions. There is no membrane depolarization between bursts of AP. (b) At II 10^{-6} M produces a maximal electrical and mechanical response: a membrane depolarization and superimposed APs is associated with a sustained contraction and superimposed phasic contractions. For (a) and (b), upper tracing: intracellular recording of electrical activity; lower tracing: mechanical activity. At II was applied at the first arrow and washed out at second arrow (W).

Action of angiotensin II on electrical and mechanical activity of portal vein

At low concentrations, At II induced an increase in the frequency of bursts of APs (Figure 2a), and, depending on the preparations, an increase in the number of spikes in a burst; when this latter effect occurred, it was correlated with an increase in the amplitude of the summated contractions associated with AP (phasic contractions, not shown). The threshold concentration for these effects was about 5×10^{-10} M. Generally there was no obvious resting membrane potential modification below a concentration of 3×10^{-9} M At II, then a concentration-dependent depolarization occurred with a superimposed increase in AP frequency. A sustained contraction and superimposed phasic contractions were associated with the membrane depolarization and AP respectively (Figure 2b).

In order to measure accurately the concentration-dependent membrane depolarization and associated contraction induced by higher concentrations of At II, we blocked the AP discharge by the use of Mn^{2+} 1 mM.

It has been shown by Mironneau & Gargouil (1979) that the ionic mechanisms responsible for AP generation in the rat portal vein are very similar to those in visceral smooth muscle. In particular, Mn^{2+}, a calcium antagonist, is able to block the inward current responsible for the upstroke of AP, suggesting a predominant role of Ca^{2+} in the development of this inward current.

The spontaneous electrical and mechanical activities ceased within 2 to 3 min of the application of Mn^{2+}. The At II concentration-response curves were studied, 10 min after the addition and in the continuous presence of the calcium antagonist (Figure 3).

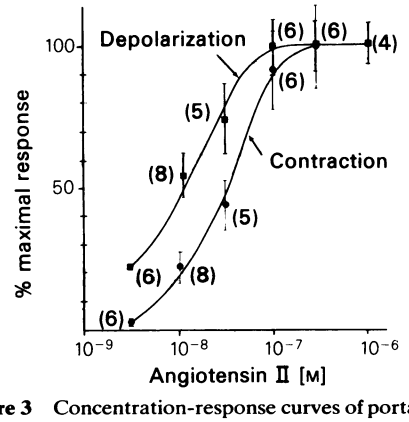

Figure 3 Concentration-response curves of portal vein to angiotensin II (At II) in the presence of 1 mM Mn^{2+}, added in order to suppress spikes (see text). (■) Depolarization curve; (●) contraction curve. The number of experiments is indicated near each point; s.e.means are indicated for each point by vertical lines.

The EC_{50} for depolarization and contraction were 1.2×10^{-8} M and 3.3×10^{-8} M respectively; the maximal responses were attained at 10^{-7} M for depolarization and 3×10^{-7} M for contraction.

Action of angiotensin II at low concentrations in normal solution

We studied the action of concentrations of At II between 5×10^{-10} and 3×10^{-9} M.

Although At II obviously synchronized the activity of the cells in the preparation, the complex pattern of bursts of spikes observed in the absence of the peptide, made it difficult to analyse the modification of the shape of AP that it induced at 37°C. Occasionally, such a study can be made on well synchronized preparations. Figure 4a shows the action of At II 3×10^{-9} M on the shape of AP at the beginning (middle trace) and at the time of its maximum effect (lower trace). There was no change in the amplitude of AP; although there was a tendency to an increase in the maximum rate of rise of the AP, no significant difference could be detected. A rebound on the plateau of repolarization and a prolongation of plateau duration were frequently observed. APs were triggered by slow spontaneous depolarizations (pacemaker potentials) originating in pacemaker regions (Hermsmeyer, 1971; 1973). The slope of these pacemaker potentials was always increased by At II.

The same analysis was easier at 25°C. At this temperature the APs were very often isolated, and consequently the associated phasic contractions rarely summated. As shown in Figure 4b, the amplitude of phasic contractions was increased by At II. There was no increase in AP amplitude, when the membrane potential was maintained at the resting level, the depolarization being compensated for by a hyperpolarizing current.

Action of angiotensin II in the presence of tetraethylammonium

In order to analyse further the action of At II on electrical and mechanical activities at low concentrations on the portal vein, we performed experiments in the presence of TEA 10 mM. TEA has been reported to block partially the potassium permeability of smooth muscle cell membrane (Casteels, Kitamura, Kuriyama & Suzuki, 1977; Haeusler, 1978). Portal vein APs have been shown to be due mainly to an inward Ca^{2+} current and an outward K^+ current (Kumamoto, Fujiwara, Muramatsu & Kajimoto, 1978; Mironneau & Gargouil, 1979). If At II activates both currents simultaneously, it is likely that an increase in AP amplitude would be more evident in the presence of a K^+ channel blocker.

TEA (10 mM) induced a slight depolarization (membrane potential decreased to 46.4 ± 0.7 mV, $n = 8$) and rhythmic activity which was maintained throughout the period of its application. The amplitude and duration of APs were increased and overshoots were very frequently observed. Under these conditions At II was still capable of accelerating spike frequency and increasing tension (Figure 5a). Al-

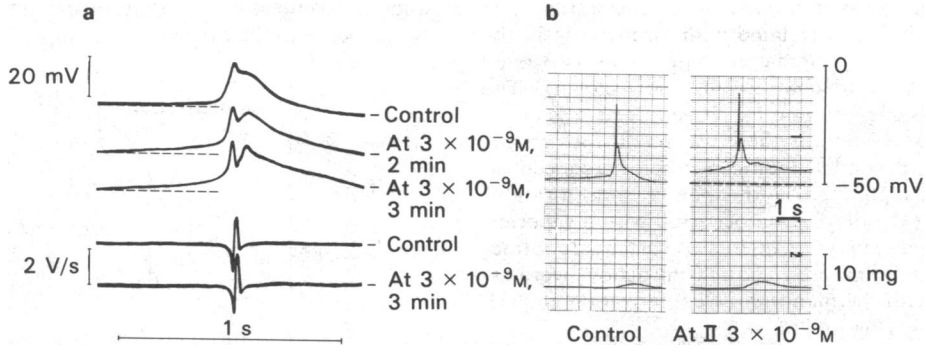

Figure 4 Typical modification of action potential (AP) configuration by angiotensin II (At II) 3×10^{-9} M. In (a) the three upper traces show, from top to bottom: AP in control solution, AP 2 min after application of At II, AP 3 min after application of At II at 37°C. The two lower traces show the rate of rise (dV/dt) of the AP under control conditions and of the AP 3 min after application of At II. The rate of the AP rise, 2 min after application of At II has been omitted for the sake of clarity. dV/dt trace is downward for depolarizations. There is no obvious modification in the amplitude or maximal rate of rise of AP, but the slope of the slow depolarization (pacemaker potential) which precedes the APs is increased during At II application (the dotted line permits the increase in the slope of pacemaker potential to be seen). The oscilloscope traces have been retouched to improve copying. In (b) modification of AP configuration and phasic contraction amplitude at 25°C. Note the acceleration of pacemaker potential and the increase in contraction amplitude. The depolarization produced by At II was not compensated on this figure. The dashed line indicates the resting potential before At II application. Chart recorder tracings.

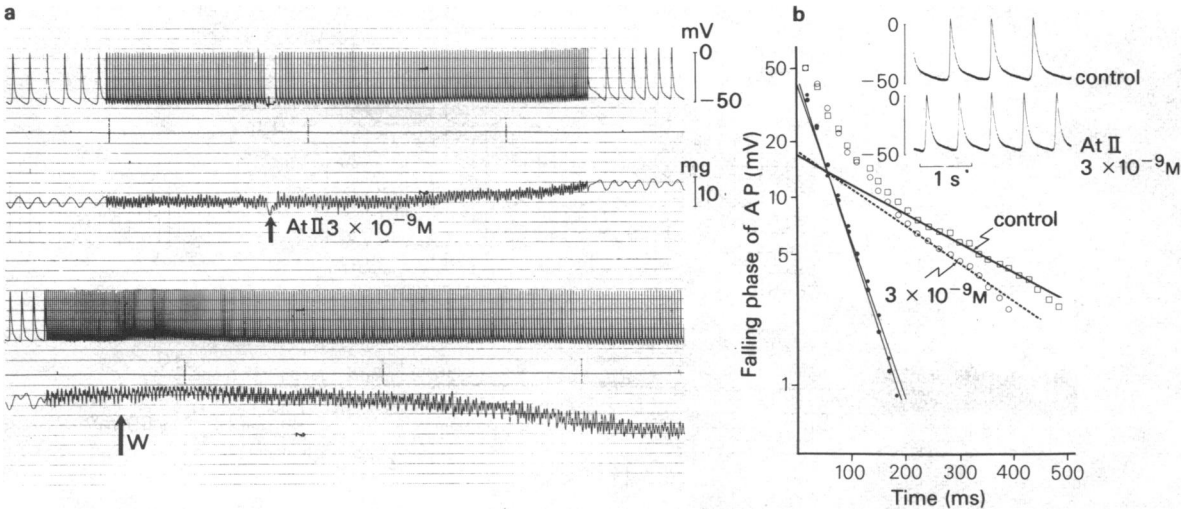

Figure 5 (a) Acceleration by angiotensin II (At II) of rhythmic activity triggered by 10 mM tetraethylammonium (TEA). TEA was introduced 25 min before the beginning and was present throughout duration of recording. The lower panel is the continuation of the upper panel with an interruption of 2 min. Two recording speeds have been used: 10 mm/s for the faster and 100 mm/min for the slower. (b) The inset shows an enlargement of APs during rhythmic activity induced by 10 mM TEA in the physiological solution (control) and 2 min 30 s after addition of At II to the TEA containing medium. Note the acceleration by At II of the repolarizing phase of AP. Oscilloscope tracings taken from the same experiment as on the left panel. A semi-logarithmic plot of the falling phase of AP in solution containing TEA (control) (□) and after addition of At II (○) is shown in the lower part. The first (fast) exponential component of each curve (●) was obtained after substraction of the second (slow) exponential component from the experimental points. Ordinate scale: falling phase of AP in mV; abscissa scale: time from the summit of AP.

though very much reduced, the pacemaker potential was still accelerated by At II (3×10^{-9} M) (Figure 5b). When the depolarization induced by At II was compensated for, neither the AP amplitude nor the rate of rise of AP were significantly affected. However, the slow phase of repolarization of AP was accelerated by the polypeptide (Figure 5b). A semilogarithmic plot of the falling phase of AP (logarithmic decrease of AP amplitude versus time) shows that the first phase of repolarization was not affected by At II but the second exponential phase was clearly accelerated.

Action of angiotensin II in the presence of ouabain

It has been suggested that the spontaneous activity of smooth muscle, is partly controlled by a metabolic component (Tomita & Watanabe, 1973) dependent on the Na-K pump (Daniel, 1965; Liu, Prosser & Job, 1969; Tomita & Yamamoto, 1971).

We decided to determine whether the increase in spontaneous activity induced by At II persisted in the presence of ouabain at concentrations which blocked the Na pump (Garay, Moura, Osborne-Pellegrin, Papadimitriou & Worcel, 1979). The addition of ouabain (10^{-3} M) to the normal perfusing medium containing 10 mM TEA produced a depolarization of 5 to 10 mV accompanied by an AP acceleration. These phenomena were reversible when ouabain was washed out.

When At II was added to a medium containing 10^{-3} M ouabain (with 10 mM TEA, in order to induce rhythmic activity), the frequency of spontaneous discharge was either unaffected or slightly reduced, the pacemaker potential always being decreased. Under these conditions, At II produced a marked increase in the amplitude of phasic contractions, which was more important than in the absence of ouabain. Figure 6a shows an example of the action of At II in Krebs solution containing 10 mM TEA and 10^{-3} M ouabain. Phasic contractions were hardly distinguishable before peptide application, due to summation, but At II 10^{-9} M, a concentration which, in the absence of ouabain did not affect these contractions, greatly increased their amplitude when the glycoside was present. This could be due to an inhibition of transmembrane Ca^{2+} extrusion through the Na-Ca exchange mechanism, secondary to the augmentation of intracellular Na^{+} concentration, resulting from the blockade of the Na-K pump by ouabain (Van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973; Brading & Widdicombe, 1975; Blaustein, 1977). Higher concentrations of ouabain (3×10^{-3} M) produced a 15 to 20 mV depolarization

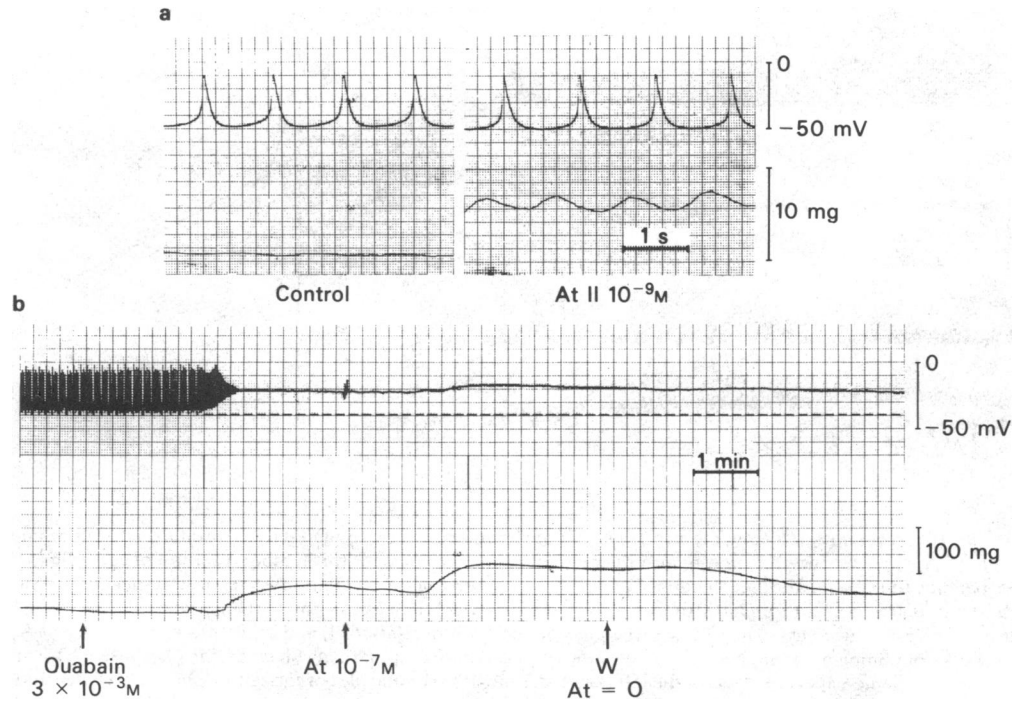

Figure 6 (a) Effect of angiotensin II (At II 10^{-9} M) on phasic activity in the presence of ouabain (10^{-3} M). Left section: activity recorded in 10 mM tetraethylammonium (TEA) + ouabain medium; right section: activity recorded in 10 mM TEA + ouabain medium, 3 min after addition of At II. Under these conditions, At II only induces an increase in the amplitude of phasic contractions (see text). (b) Effect of ouabain (3×10^{-3} M) on the tonic electrical and mechanical responses of portal vein to At II (10^{-7} M). TEA (10 mM) was present throughout the experiment. Ouabain was applied at the first arrow and was present in the perfusing medium during the rest of the experiment. The dashed line indicates the resting membrane potential in the TEA medium before application of ouabain. In the presence of this concentration of ouabain, At II (10^{-7} M) produces a tonic contraction, even if the membrane potential is barely modified, and in the absence of AP discharge.

and a blockade of AP discharge. Under these conditions, even a concentration of At II as high as 10^{-7} M had a minimal depolarizing effect. Nonetheless, this peptide concentration still induced a contraction (Figure 6b), larger than the contractions observed in the absence of glycoside.

All these effects of ouabain were not inhibited by tetrodotoxin (3×10^{-6} M) or phentolamine (10^{-5} M).

Discussion

Our results confirm the great sensitivity of the rat portal vein to At II (Bohr & Uchida, 1967; Carruba *et al.*, 1973), since an increase in the rhythmic and mechanical activities could be detected with concentrations as low as 5×10^{-10} M. It is interesting to note that the threshold response of the portal vein is obtained at concentrations of the same order of magnitude as the circulating blood levels (Powell-Jackson & McGregor, 1976).

At low concentrations (between 5×10^{-10} M and 10^{-9} M) At II induces an acceleration of AP discharge without modifying the resting membrane potential. The normal spontaneous electrical activity appears to originate from pacemaker cells (Hermsmeyer, 1971; 1973). At II increases the slope of this pacemaker potential, as a consequence the AP triggering threshold is attained more rapidly. The Na-K pump may be involved in the acceleration of AP discharge rate produced by At II, since this effect disappears in the presence of 1 mM ouabain.

The acceleration of the pacemaker potential could also be explained by a reduction of the deactivation time constant for a K^+ current, unmasking a background inward current as proposed by Brown, Giles & Noble (1977) and Lenfant, Mironneau & Aka (1972) for the heart and Mironneau (1976) for uterine smooth muscle. This mechanism seems also responsible for the rhythmic activity induced by At II on the rat uterus (Mironneau, Mironneau, Grosset, Hamon & Savineau, 1980).

In experiments performed in the presence of 10 mM TEA, At II accelerates the repolarizing phase of AP. Since this AP component is mainly due to an increase in potassium conductance (g_{K^+}) (Kumamoto *et al.*, 1978; Mironneau & Gargouil, 1979), it may be suggested that At II activates a voltage-dependent K^+ channel. In fact, such an effect on g_{K^+} has been observed under voltage-clamp conditions with high concentrations of the peptide (Mirronneau & Gargouil, 1979). This At II action could be primary or secondary to an increase in intracellular Ca^{2+} concentration; indeed, it has been suggested that outward currents in the rat myometrium may be activated by this mechanism (Vassort, 1975; Mironneau & Savineau, 1980).

Three pharmacologically distinct K^+ channels have been observed in molluscan neurones (Thompson, 1977). The participation of K^+ channels in the action of At II remains possible under our experimental conditions, if more than one type of these channels is similarly postulated in the rat portal vein. Moreover, TEA at a concentration of 10 mM, only partially inhibits voltage-dependent potassium channels.

At II at low concentrations produces an increase in phasic contraction amplitude with no detectable change in the maximal rate of rise or size of the associated AP. This action of the peptide is very probably due to an increased release of Ca^{2+} from intracellular stores. Indeed, it has been suggested that Ca^{2+} entry during the upstroke of AP may not be sufficient to generate a full contraction and a Ca^{2+}-induced Ca^{2+} release could be a mechanism contributing to tension generation (Bolton, 1979). Furthermore, Ca^{2+} release from intracellular stores could be activated directly following At II-receptor binding, by pharmacomechanical coupling (Somlyo & Somlyo, 1968; Johansson & Somlyo, 1980).

At concentrations higher than 3×10^{-9} M, At II triggers additional electrical and mechanical phenomena. There is a concentration-dependent depolarization (associated with an increasingly high spike discharge), accompanied by a concentration-dependent contraction. Undoubtedly, under these conditions, APs must contribute to the activation of contractile mechanisms. Nonetheless, the experiments performed in the presence of 1 mM Mn^{2+} suggest strongly that At II at higher concentrations may also induce either a primary increase in Ca^{2+} conductance ($g_{Ca^{2+}}$) through receptor-operated channels, or a release of Ca^{2+} from intracellular stores. Even if Mn^{2+}, at 1 mM is able to block the activation of a voltage-dependent $g_{Ca^{2+}}$ (spikes are suppressed), it is not possible to ensure that other Ca^{2+} channels or Ca^{2+} exchange mechanisms are inactivated under these experimental conditions. However, we have shown previously that At II at high concentrations, does not activate receptor-operated Ca^{2+} channels directly in rat myometrium (Hamon & Worcel, 1979). By analogy, it may be assumed that concentration-dependent tonic contraction associated with the depolarization induced by At II in the presence of Mn^{2+}, is due mainly to the release of Ca^{2+} from intracellular stores. This phenomenon may be triggered by membrane depolarization and/or pharmacomechanical coupling. The importance of this latter mechanism is further suggested by the effects of At II 10^{-7} M in the presence of 3 mM ouabain, since under these conditions, the peptide is able to induce a strong tonic contraction at the same time as a very slight depolarization.

In conclusion, At II activates different excitation-contraction coupling mechanisms in vascular smooth muscle. At low, physiologically relevant concentrations, the peptide induces mainly an acceleration of AP discharge, acting on the pacemaker potential, through a mechanism dependent on the Na-K pump. At the same time, Ca^{2+} release may be activated by pharmacomechanical coupling.

At higher concentrations, At II induces a membrane depolarization and bursts of spikes. The associated contraction appears to be activated by the secondary increase in Ca^{2+} influx (through the increase in the number of APs) and pharmacomechanical coupling.

Furthermore, Mironneau & Gargouil (1979) using the voltage-clamp technique in rat myometrium, have reported the activation of voltage-dependent $g_{Ca^{2+}}$ by high concentrations of At II (10^{-7} M). We are not able to confirm or exclude this possible additional effect of At II under our experimental conditions.

References

AXELSSON, J., WAHLSTRÖM, B., JOHANSSON, B. & JONSSON, O. (1967). Influence of the ionic environment on spontaneous electrical and mechanical activity of the rat portal vein. *Circulation Res.*, **21**, 609–618.

BLAUSTEIN, M.P. (1977). Sodium ions, calcium ions, blood pressure regulation and hypertension: a reassessment and a hypothesis. *Am. J. Physiol.*, **232**, C165–C173.

BOHR, D.F. & UCHIDA, E. (1967). Individualities of vascular smooth muscles in response to angiotensin. *Circulation Res.*, **21**, Suppl. II, 135–143.

BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

BRADING, A.F. & WIDDICOMBE, J.H. (1975). Interaction

between sodium and calcium movements in smooth muscle. *INSERM Colloq.*, **50,** 235–246.

BROWN, H.F., GILES, W. & NOBLE, S.J. (1977). Membrane currents underlying activity in frog sinus venosus. *J. Physiol.*, **271,** 783–816.

BÜLBRING, E. (1953). Measurements of oxygen consumption in smooth muscle. *J. Physiol.*, **122,** 111–134.

CARRUBA, M., MANDELLI, V. & MANTEGAZZA, P. (1973). The effect of angiotensin II and other vasoactive drugs on isolated portal vein preparations. *Archs int. Pharmacodyn. Thér.*, **201,** 224–233.

CASTEELS, R., KITAMURA, K., KURIYAMA, H. & SUZUKI, K. (1977). The membrane properties of the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.*, **271,** 41–61.

CUTHBERT, A.W. & SUTTER, M.C. (1964). Electrical activity of a mammalian vein. *Nature*, **202,** 95.

DANIEL, E.E. (1965). Effects of intra-arterial perfusions on electrical activity and electrolyte contents of dog small intestine. *Can. J. Physiol. Pharmac.*, **43,** 551–577.

FUNAKI, S. & BOHR, D.F. (1964). Electrical and mechanical activity of isolated smooth muscle of the rat. *Nature*, **203,** 192–194.

GARAY, R.P., MOURA, A.M., OSBORNE-PELLEGRIN, M.J., PAPADIMITRIOU, A. & WORCEL, M. (1979). Identification of different sodium compartments from smooth muscle cells, fibroblasts and endothelial cells, in arteries and tissue culture. *J. Physiol.*, **287,** 213–229.

GRÄNDE, P.O. (1979). Dynamic and static components in the myogenic control of vascular tone in cat skeletal muscle. *Acta physiol. scand.*, Suppl. **476,** 1–44.

HAEUSLER, G. (1978). Relationship between noradrenaline-induced depolarization and contraction in vascular smooth muscle. *Blood Vess.*, **15,** 46–54.

HAMON, G. & WORCEL, M. (1979). Electrophysiological study of the action of angiotensin II on the rat myometrium. *Circulation Res.*, **45,** 234–243.

HERMSMEYER, K. (1971). Contraction and membrane activation in several mammalian vascular muscles. *Life Sci.*, **10,** 223–234.

HERMSMEYER, K. (1973). Multiple pacemaker sites in spontaneously active vascular muscle. *Circulation Res.*, **33,** 244–251.

JOHANSSON, B. & SOMLYO, A.P. (1980). Electrophysiology and excitation-contraction coupling. In *Handbook of Physiology. The Cardiovascular System*, Vol. II. ed. Bohr, D.F., Somlyo, A.P. & Sparks, H.V. Jr. pp. 301–323. Bethesda, M.D.: Am. Physiol. Soc.

KAO, C.Y. & NISHIYAMA, A. (1964). Ovarian hormones and resting potentials of uterine smooth muscle. *Am. J. Physiol.*, **207,** 793–799.

KUMAMOTO, M., FUJIWARA, M., MURAMATSU, I. & KAJIMOTO, N. (1978). Ionic mechanisms of membrane excitability of the rat portal vein. *Blood Vess.*, **15,** 209–210.

LENFANT, J., MIRONNEAU, J. & AKA, J.K. (1972). Activité répétitive de la fibre sino-auriculaire de grenouille: analyse des courants membranaires responsables de l'automatisme cardiaque. *J. Physiol. (Paris)*, **64,** 5–18.

LIU, J., PROSSER, C.L. & JOB, D.D. (1969). Ionic dependence of slow waves and spikes in intestinal muscle. *Am. J. Physiol.*, **217,** 1542–1547.

LJUNG, B. (1970). Nervous and myogenic mechanisms in the control of a vascular neuroeffector system. *Acta physiol. scand.*, Suppl. **349,** 33–68.

LUSAMVUKU, N.A.T., SERCOMBE, R., AUBINEAU, P. & SEYLAZ, J. (1979). Correlated electrical and mechanical responses of isolated rabbit pial arteries to some vasoactive drugs. *Stroke*, **10,** 727–732.

MIRONNEAU, J. (1976). Effects of oxytocin on ionic currents underlying rhythmic activity and contraction in uterine smooth muscle. *Pflügers Arch.*, **363,** 113–118.

MIRONNEAU, J. & GARGOUIL, Y.M. (1979). Action of indapamide on the excitation-contraction coupling in vascular smooth muscle. *Eur. J. Pharmac.*, **57,** 57–67.

MIRONNEAU, J., MIRONNEAU, C., GROSSET, A., HAMON, G. & SAVINEAU, J.P. (1980). Action of angiotensin II on the electrical and mechanical activity in rat uterine smooth muscle. *Eur. J. Pharmac.*, **68,** 275–285.

MIRONNEAU, J. & SAVINEAU, J.P. (1980). Effects of calcium ions on outward membrane currents in rat uterine smooth muscle. *J. Physiol.*, **302,** 411–425.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circulation Res.*, **41,** 19–26.

POWELL-JACKSON, J.D. & McGREGOR, J. (1976). Radioimmunoassay of angiotensin II in the rat. *J. Endocrinol.*, **68,** 175–176.

REGOLI, D., RIOUX, F. & PARK, W.K. (1974). Pharmacology of angiotensin. *Pharmac. Rev.*, **26,** 69–123.

SOMLYO, A.P. & SOMLYO, A.V. (1970). Electrophysiological correlates of the inequality of maximal vascular smooth muscle contractions elicited by drugs. In *Vascular Neuroeffector Systems.* ed. Bevan, J.A., Furchgott, R.F., Maxwell, R.A. & Somlyo, A.P. pp. 216–228. Basel: S. Karger.

SOMLYO, A.V. & SOMLYO, A.P. (1968). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *J. Pharmac. exp. Ther.*, **159,** 129–145.

THOMPSON, S.H. (1977). Three pharmacologically distinct potassium channels in molluscan neurons. *J. Physiol.*, **265,** 465–488.

TOMITA, T. & WATANABE, H. (1973). Factors controlling myogenic activity in smooth muscle. *Phil. Trans. R. Soc.*, B **265,** 73–85.

TOMITA, T. & YAMAMOTO, T. (1971). Effects of removing the external potassium on the smooth muscle of the guinea-pig taenia-coli. *J. Physiol.*, **212,** 851–868.

VAN BREEMEN, C., FARINAS, B.R., CASTEELS, R., GERBA, P., WUYTACK, F. & DETH, R. (1973). Factors controlling cytoplasmic Ca^{2+} concentration. *Phil. Trans. R. Soc.*, B, **265,** 57–71.

VASSORT, G. (1975). Voltage-clamp analysis of transmembrane ionic currents in guinea-pig myometrium: evidence for an initial potassium activation triggered by calcium influx. *J. Physiol.*, **252,** 713–734.

WESTON, A.H. & GOLENHOFEN, K. (1976). Comparison of the excitatory and inhibitory effects of angiotensin and vasopressin on mammalian portal vein. *Blood Vess.*, **13,** 350–360.

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