Transient outward current in adult rat supraoptic neurones with slice patch-clamp technique: inhibition by angiotensin II

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1. Outward potassium currents were recorded from microscopically identified supraoptic neurones of adult Wistar male rats using the whole-cell patch-clamp technique in thin-slice preparations. The basic characteristics of transient outward current (I_A or A-current) and the effects of angiotensin II (AII) on the currents were studied.

2. I_A was isolated by subtracting outward currents elicited by stepping from two different holding potentials to a test potential or by applying 4-aminopyridine (4-AP) at 5 mM. The isolated I_A had a threshold for activation between −55 and −65 mV and was characterized by fast activation and inactivation. Values of the time to peak and the inactivation time constants for current decay at different test potentials were voltage dependent.

3. Normalized currents for activation and steady-state inactivation of I_A were fitted to the Boltzmann function. The mid-points and the slope factors were, respectively, −35·0 and −14·3 ± 0·40 mV ($n = 5$) for the activation curve, and −72·0 and 7·0 ± 0·68 mV ($n = 5$) for the inactivation curve.

4. The time course of recovery from inactivation was best fitted to a single exponential function with the time constant of 37·8 ± 6·6 ms ($n = 6$).

5. The effects of AII on I_A and delayed rectifier current (I_K) were investigated. According to their responses to AII, cells were classified into two groups, sensitive and low-sensitive. Bolus injection of AII (10 μM, 100 μl) decreased the I_A amplitude by 25·1 ± 2·4% in seven (53·8%) of the thirteen neurones tested (sensitive group), whereas the other six neurones (low-sensitive group) changed by only 2·2 ± 0·8%. Perfusion of AII (0·1 μM) decreased the I_A amplitude by 21·3 ± 3·1% in six (54·5%) of eleven neurones tested (sensitive group), whereas the other five neurones (low-sensitive group) changed only by 1·7 ± 0·8%. Bolus injection of AII (10 μM, 100 μl) decreased the I_K amplitude 9·6 ± 1·6% mV in five (45·5%) of the eleven neurones tested (sensitive group), whereas the other six neurones (low-sensitive group) changed only by 0·46 ± 0·27%. In the sensitive groups, the reduction of I_A by AII was significantly larger than that of I_K ($P < 0·05$).

6. Application of saralasin at 1 μM, an AII antagonist, blocked the effects of AII on I_A.

7. These results suggest that the excitatory action of AII on supraoptic neurosecretory cells is mediated at least in part through suppression of I_A.

Arginine vasopressin (AVP) and oxytocin (OXT) are well known as posterior pituitary hormones which control body fluid and milk ejection, respectively. The peptides are synthesized mainly in neurones of the supraoptic (SON) and paraventricular (PVN) nuclei, and released from their axon terminals located in the neurohypophysis. The amount of hormone released is closely related to the firing activity of the neurosecretory cells in the SON and PVN and the activity of the cells may be modified by both neuronal and humoral signals (Poulain & Wakerley, 1982).

Three classes of voltage-dependent potassium currents have been reported in SON neurones: the delayed rectifier, the transient outward and the Ca^{2+}-activated potassium currents (Cobbett, Legendre & Mason, 1989). The transient outward current (I_A or A-current) is known to exist in most excitable cells, and is thought to be activated by the hyperpolarizing after-potential and to regulate spike frequency by delaying the next spike (Connor & Stevens, 1971; Rogawski, 1985; Rudy, 1988). The I_A of SON neurones has already been studied using cultured neurones (Cobbett *et al.* 1989; Müller, Misgeld &

Swandulla, 1992) and perfused explants (Bourque, 1988). However, little is known about modulation of I_A by neurotransmitters and neuromodulators in the SON.

Angiotensin II (AII) has several central actions; it increases both AVP and OXT release, induces drinking behaviour, enhances salt appetite and increases blood pressure (Phillips, 1987). AII immunoreactive neurones and terminals, and AII receptors have been found in several regions of the brain including the SON (Mendelsohn, Quirion, Saavedra, Aguilera & Catt, 1984; Lind, Swanson & Ganten, 1985). We have already reported that AII increases the firing rate of SON neurones *in vitro* (Okuya, Inenaga, Kaneko & Yamashita, 1987) and have suggested that AII acts as a neurotransmitter or neuromodulator in the SON (Yamashita & Kannan, 1992).

There are several reports that AII affects channels in cell membranes. It reduces potassium currents in single aortic cells of rabbit (Bkaily *et al.* 1988), zona glomerulosa cells of rat, bovine and human (Brauneis, Vassilev, Quinn, Williams & Tillotson, 1991) and renal juxtaglomerular cells of mouse (Kurtz & Penner, 1989).

To elucidate the mechanism of the excitatory action of AII on SON neurones, I_A was isolated from other outward potassium currents. Both the basic characteristics of I_A and effects of AII on this current were investigated using thin slice preparations of rat brain and the whole-cell patch-clamp technique.

METHODS

Slice preparation

Young adult male Wistar rats weighing 150–240 g were stunned by a blow on the back of the neck and rapidly decapitated. The brains were quickly removed and were cooled in a bathing medium at 4 °C for approximately 1 min. A block containing the hypothalamus was cut from the brain and was glued to the stage of a vibratome-type slicer (DSK-2000; DSK, Kyoto, Japan). After the meninges were carefully removed, coronal slices 120–140 μm in thickness were cut from the block in bathing medium at 4 °C. The slices were carefully trimmed with a circular punch (i.d. 1·8 mm). Immediately after trimming, the slices were pre-incubated in the bathing medium at room temperature and left for at least 1 h until they were transferred to the recording chamber.

Solutions and drugs

The incubation medium for slice preparations contained (mM): NaCl, 124; KCl, 5; KH_2PO_4, 1·24; $MgSO_4$, 1·3; $CaCl_2$, 2·1; $NaHCO_3$, 20; and glucose, 10. A perfusion medium was used containing (mM): choline chloride, 135; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 2; $CoCl_2$, 2; Hepes, 10; and glucose, 10. To block Na^+ current, this medium did not contain Na^+ and $CoCl_2$ was added to block Ca^{2+} current and Ca^{2+}-activated K^+ current. The medium was adjusted to pH 7·4 with Tris base. The osmolality of the perfusion medium was 284 mosmol kg^{-1}. In some experiments, tetraethylammonium (TEA) chloride, 4-aminopyridine (4-AP) and picrotoxin (Nacalai Chemicals Ltd, Kyoto, Japan) were added to the perfusion medium. All of the bathing and perfusion media used in this experiment were oxygenated with a mixture of 95% O_2–5% CO_2. The pipette solution used in the recording electrodes contained (mM): KCl, 140; $MgCl_2$, 1; $CaCl_2$, 1; EGTA, 10; and Na-ATP, 2 (pH 7·3 adjusted with Tris base). Angiotensin II (AII) and [Sar^1, Ala^8]-angiotensin II (saralasin) were commercially purchased from Peptide Institute, Mionh, Japan.

Fixation of the slice in the recording chamber and microscopic identification of SON neurones

The slice preparations were fixed in the recording chamber as previously described (Edwards, Konnerth, Sakmann & Takahashi, 1989; Konnerth, 1990). Slices were placed in the glass-bottomed recording chamber and fixed with a grid of parallel nylon threads supported by a U-shaped platinum weight. The volume of the recording chamber was 1·0 ml and the flow rate of the perfusion medium was adjusted to 2 ml min^{-1}. The solution level was kept constant by a low-pressure aspiration system.

An upright microscope (Nikon, Tokyo) with Nomarski optics in which the focusing mechanism had been altered to move the objectives instead of the stage was used to observe the upper surface of the cells. The objectives were a ×4 dry lens and a ×40 water immersion lens with a working distance of 1·6 mm. We could easily distinguish putative magnocellular neurones in the SON from glia and the other structures by observing their size, shape and the number of dendrites.

Recordings and data analysis

The electrodes used in this experiment were double-pulled (PP-83; Narishige, Tokyo) from thick-wall borosilicate glass and polished (MF-83; Narishige, Tokyo). They had a final resistance between 4 and 6 MΩ when filled. They were coated except at the tip with nail enamel to reduce the capacitance resulting from deep immersion of the pipettes in the bath.

Electrophysiological recordings were carried out at room temperature of 25 °C. Whole-cell tight-seal (> 5 GΩ) recordings were made from microscopically identified SON neurones in the upper surface of the slices. They were characterized by their bright appearance under phase contrast (×400) and their spherical or bipolar shapes with a small number of processes.

Currents were recorded with an EPC-7 patch-clamp amplifier (List Electronic, Darmstadt, Germany). Signals were digitized with an analog–digital converter (Model ADX-98E, Canopus, Kobe), and monitored and stored with a microcomputer on-line for subsequent off-line analysis. The signals were also stored on videotape, through a pulse code modulator (Sony Co., Tokyo). Data acquisition, current averaging, subtraction of capacitance and linear leakage currents were performed using a suite of programs (QP-120J; Nihon Kohden, Tokyo). Current records were corrected for leakage and capacitative currents by subtracting the scaled currents resulting from 10 mV hyperpolarizing pulses applied from the holding potential, except where otherwise described. Series resistance in whole-cell configuration was approximately 5 MΩ and compensated.

The values in this text are expressed as means ± S.E.M., except where otherwise noted and n represents the number of neurones tested. Both the comparisons between sensitive and low-sensitive groups and the comparison between I_A and I_K were made by using the Mann-Whitney U test. A P value of $< 0·05$ was considered statistically significant.

RESULTS

Whole-cell voltage-clamp recordings were made from seventy-one microscopically identified SON neurones in thin-slice preparations. The neurones recorded had a longitudinal diameter of more than 25 μm. The neurones had either a bipolar or a spherical shape with a few dendrites. Twenty-three of the seventy-one neurones were photographed and their diameters were measured precisely. The average value was $32{\cdot}3 \pm 4{\cdot}9$ μm (mean $\pm$ S.D., $n = 23$). In the current-clamp mode, the mean value of resting membrane potentials was $-54{\cdot}4 \pm 5{\cdot}9$ mV ($n = 71$).

Isolation of transient outward current

We used a protocol similar to that described by Connor & Stevens (1971) and Cobbett *et al.* (1989) to elicit and isolate outward currents. The outward current elicited by

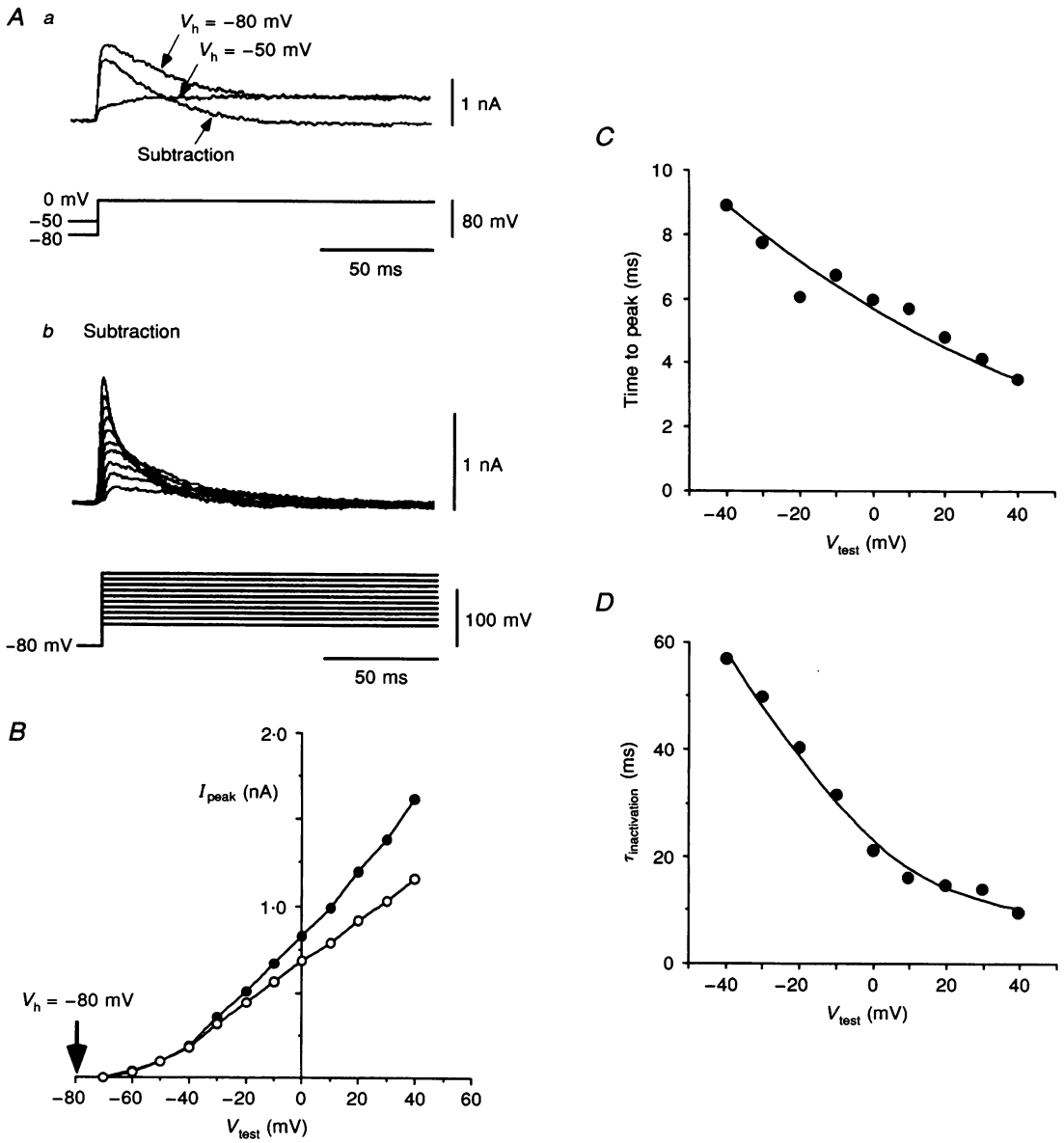

Figure 1. Isolation of a transient outward current (I_A) from total outward current

A a, two outward currents obtained by voltage steps to 0 mV from holding potentials (V_h) of −50 and −80 mV together with the isolated transient current. The isolated (subtraction) current was obtained by subtracting the current elicited by a voltage step to 0 mV from $V_h = -50$ mV from the current elicited by a step from $V_h = -80$ mV. *A b*, isolated I_A obtained by different voltage steps from $V_h = -80$ mV. Lower traces in *A a* and *b* show the voltage steps. *B*, current (I_{peak})–voltage (V_{test}) relationship in the peak outward currents obtained from records in *A*. ●, the outward currents obtained at different test potentials from $V_h = -80$ mV. ○, the isolated outward currents by subtraction. *C*, the time to peak of I_A at different test potentials. *D*, the inactivation time constant ($\tau_{inactivation}$) for current decay. Continuous lines in *C* and *D* were traced using an exponential curve fitting program.

the step from −80 mV consisted of a transient component and a maintained component but the outward current elicited by the step from −50 mV consisted mainly of the maintained component (Fig. 1*A a*). Subtraction of the two outward currents revealed the transient component. This 'subtraction current' was regarded as the isolated transient outward current, I_A. The subtraction currents at different test potentials were characterized by fast activation and inactivation (Fig. 1*A b*). The threshold membrane potential was approximately −60 mV (Fig. 1*B*). The value of the time to peak changed in a voltage-dependent manner, ranging from 8·9 ms at −40 mV to 3·5 ms at +40 mV (Fig. 1*C*). The time-dependent inactivation of the current decay fitted a single exponential function. The inactivation time constants were voltage dependent and declined exponentially from 57·0 ms at −40 mV to 9·5 ms at +40 mV (Fig. 1*D*).

Pharmacology of transient outward current

The effects of 4-AP on outward potassium currents were investigated in seven SON neurones. Addition of 5 mM 4-AP suppressed the transient component of the outward current, but did not affect the maintained component at the end of the 100 ms voltage step (Fig. 2*A a* and *b*). Subtraction of the currents before and after perfusion of 5 mM 4-AP revealed the transient component (Fig. 2*A c*). The shape of the current subtracted and the threshold membrane potential were similar to that described in Fig. 1*A b*.

To discriminate I_A pharmacologically from the whole outward potassium currents, TEA was used. The effects of 5 mM TEA on outward potassium currents were investigated in eleven SON neurones. Figure 2*B* shows a representative experiment. The effects of TEA on the outward currents were measured at the peak and at the end of the 150 ms voltage step. TEA suppressed the maintained component more than the transient component. The steady current decreased by 41·9 ± 4·4% at +30 mV while the peak current decreased by 20·0 ± 3·6% at the same potential ($n = 11$). The steady current from a holding potential of −40 mV in the same perfusion medium also decreased by 43·2 ± 3·6% at +30 mV ($n = 3$, data not shown). Application of TEA at 5 mM did not suppress the delayed rectifier current completely but allowed the I_A to be seen more clearly.

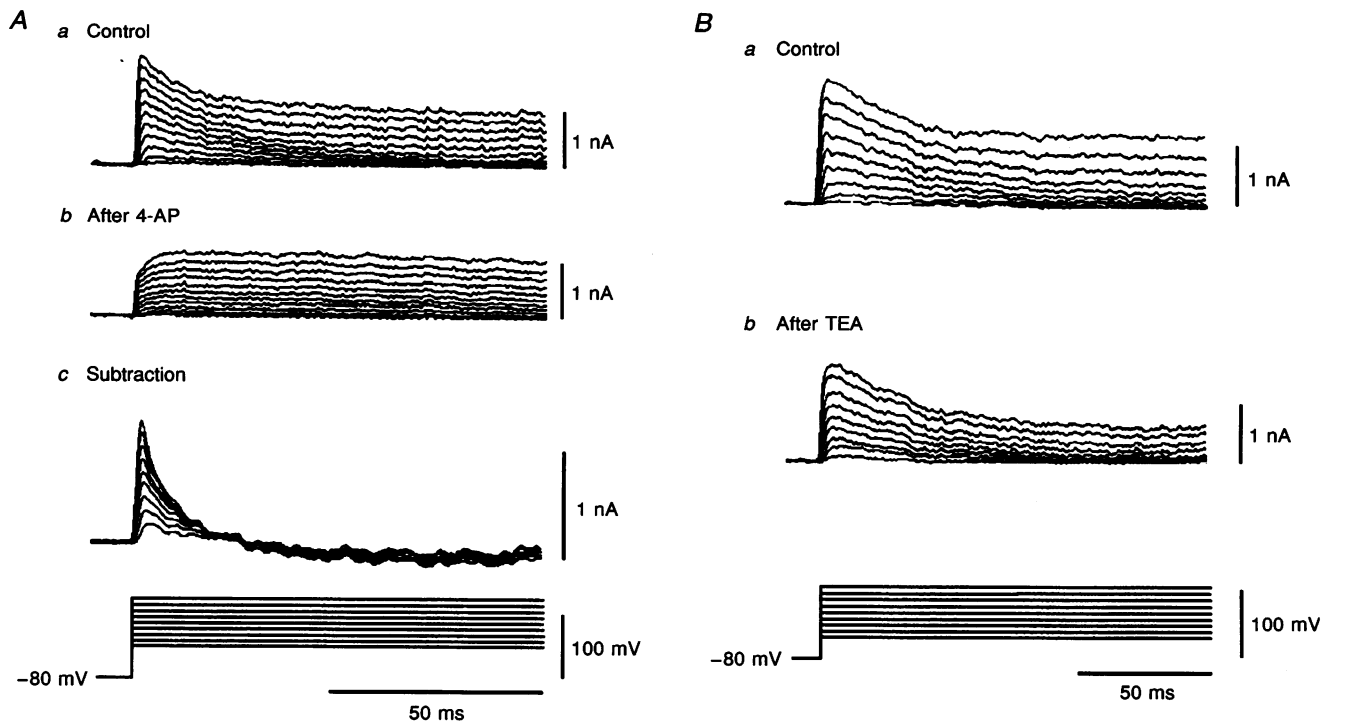

Figure 2. Pharmacological properties of the transient outward current

A a and *b*, outward currents obtained at different test potentials from $V_h = -80$ mV before (*a*) and after (*b*) 5 mM 4-AP application. *A c*, subtraction currents were obtained by subtracting records in *b* from those in *a*. *B*, the outward currents obtained at different test potentials from $V_h = -80$ mV before (*a*) and after (*b*) 5 mM TEA application. In this neurone, the steady current and the peak current decreased by 46·2 and 24·3% at +30 mV, respectively. Traces at the bottom of *A* and *B* show the voltage steps.

Activation and steady-state inactivation of transient outward current

Figure 3*A* shows activation and steady-state inactivation of I_A. The activation of I_A was obtained by delivering 10 mV step pulses at various test potentials from a holding potential of −100 mV, every 10 s. The peak conductance was calculated by dividing the peak current by the driving force. The driving force was obtained by subtracting the reversal potential from the test potential. The reversal potential was estimated from tail currents using a double voltage-step protocol (Bourque, 1988). The estimated reversal potential was −70 mV ($n = 5$). The peak conductances were normalized to the largest peak conductance. The relationship between the normalized peak conductances and test potentials fitted to a Boltzmann function of the following form:

$$g/g_{max} = (1 + \exp(V - V_{½})/\kappa^{-1},$$

with g_{max} being the maximal conductance, $V_{½}$ being the half-activation voltage and κ being the slope factor. The values of $V_{½}$ and κ were −35·0 mV and −14·3 ± 0·53 mV ($n = 5$), respectively.

The steady-state inactivation was also examined. Peak currents were measured at 0 mV from different holding potentials in 10 mV increment between −120 mV and −40 mV. The peak currents were normalized to the largest peak current. The steady-state inactivation curve was fitted to a Boltzmann function. The values of $V_{½}$ and κ were −72·0 mV and 7·0 ± 0·68 mV ($n = 5$), respectively.

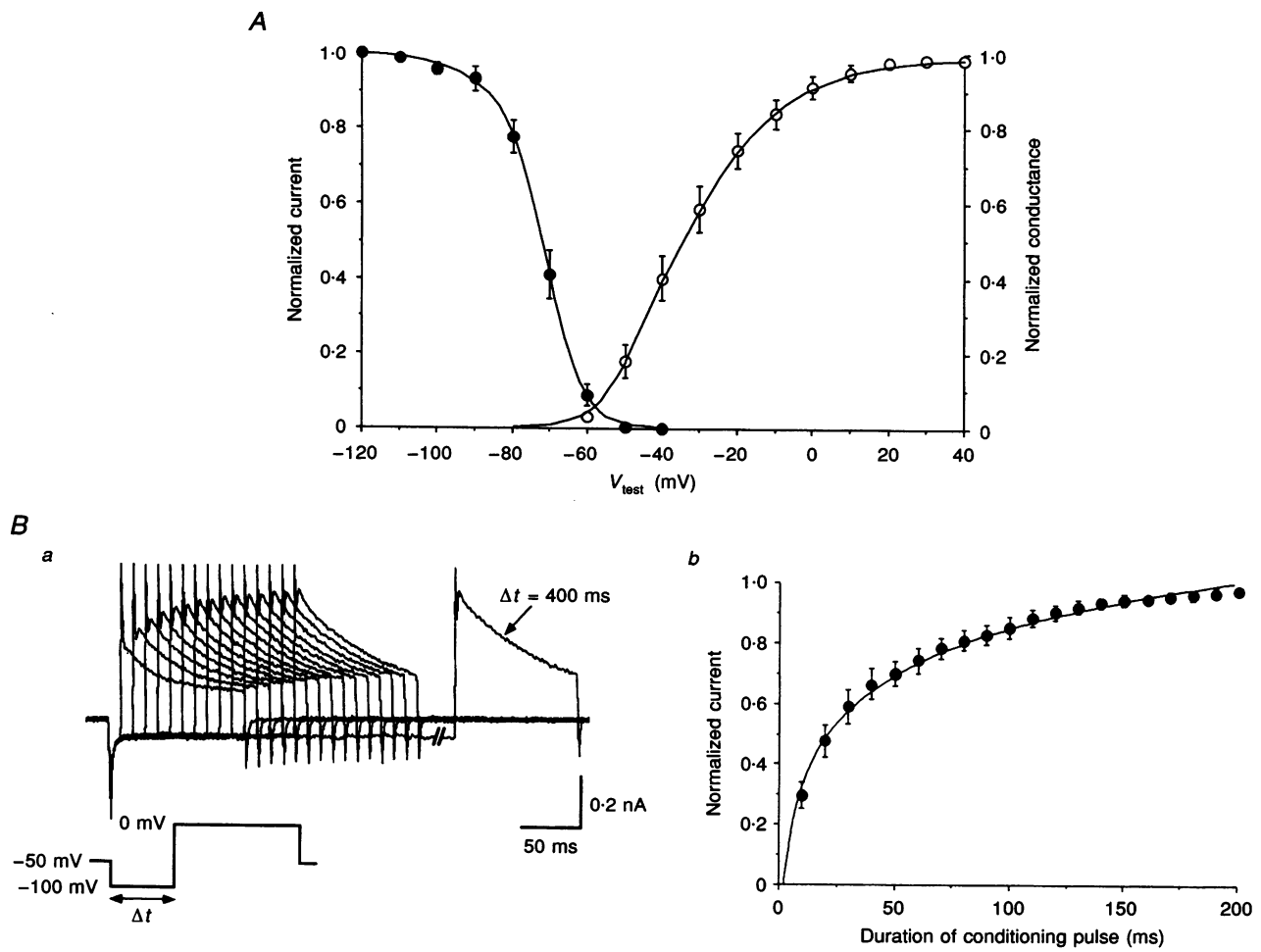

Figure 3. Kinetics of the transient outward current

A, activation (○) and steady-state inactivation (●) curves of the I_A. The experimental procedures used to obtain these curves are described in the text. Each point represents the mean ± S.E.M. obtained from five neurones. Note that the inactivation and activation curves crossed near the resting membrane potential. *Ba*, change of transient outward current with different duration of conditioning pulses. The perfusion medium contained 10 mM TEA to block the I_K. Note that the amplitude of the outward current at 150 ms of conditioning pulse is the same as that at 400 ms. *Bb*, the time course of recovery from inactivation obtained from six neurones. Each point represents the mean ± S.E.M. The normalized currents were plotted against the duration of the conditioning pulse.

Recovery of transient outward current from inactivation

The recovery of I_A from inactivation was investigated using a double voltage-step protocol. Figure 3*Ba* shows an example experiment. To test the recovery from inactivation, the neurone was held at −50 mV, at which I_A was inactivated almost completely, and 100 ms test pulses to 0 mV were delivered following hyperpolarizing conditioning pulses to −100 mV with different durations. The amplitude of I_A increased with the conditioning pulse and reached a maximum level between 150 and 200 ms. I_A was estimated by subtracting the current at the end of

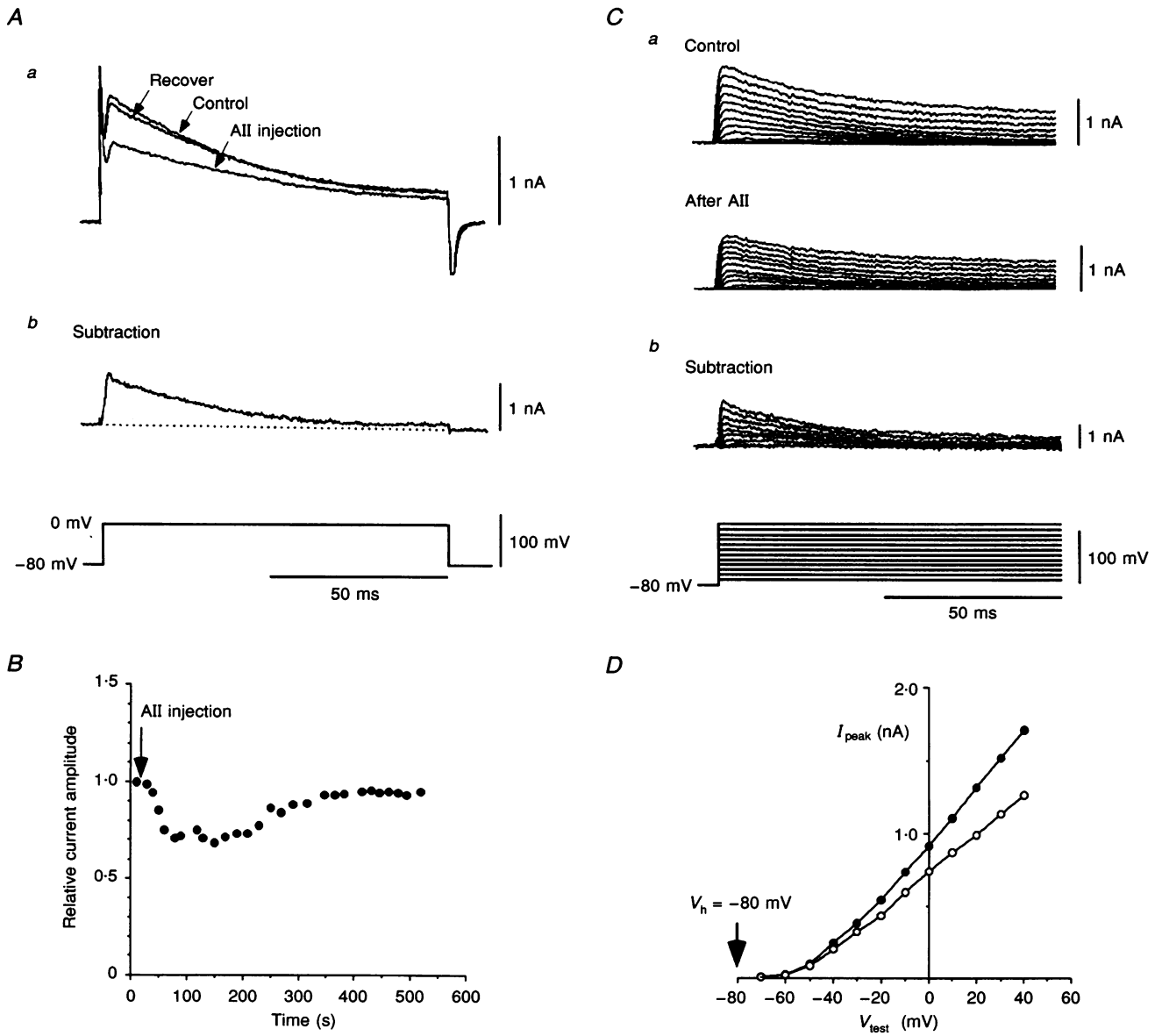

Figure 4. Effects of AII on outward currents

A a, the peak outward current was suppressed by bolus injection of AII (10 μM, 100 μl). The outward currents were elicited by a step pulse to 0 mV from $V_h = -80$ mV. I_K was suppressed by 5 mM TEA to make I_A easier to discriminate. *A b*, the trace obtained by subtracting AII-induced current from control current. Note that AII substantially decreased the I_A and partly reduced the delayed component. *B*, the change of peak currents with application of AII. The vertical axis indicates the ratio of the peak current to the control. *Ca*, perfusion of AII at 0·1 μM also suppressed the transient component in the same neurone as in *A*. The outward currents were elicited by voltage steps to different test potentials from $V_h = -80$ mV. *Cb*, currents obtained by subtraction also show that AII mainly decreased I_A. *D*, current (I_{peak})–voltage (V_{test}) relationship of peak outward current. ● and ○ show the currents before and after application of AII, respectively. In this neurone, injection of AII (10 μM, 100 μl) and perfusion of AII at 0·1 μM suppressed the peak outward current at 0 mV by 31·9 and 19·7%, respectively.

a 100 ms test pulse from the peak current in medium containing 10 mM TEA. The current was normalized to the trace following a 400 ms conditioning pulse to −100 mV. Figure 3*Bb* shows the change of the normalized current with the duration of the conditioning pulse. The time course of the recovery fitted a single exponential function with a time constant of 37·8 ± 6·6 ms ($n = 6$).

Inhibition of transient outward current by angiotensin II

Effects of AII on outward potassium currents were investigated in twenty-four SON neurones. AII was applied either by bolus injection (10 μM, 100 μl) to the chamber or by perfusion (0·1 μM). The estimated maximum concentration of AII with the bolus injection was approximately 1 μM because the volume of the chamber was 1·0 ml. The outward currents were elicited either by a voltage step to 0 mV from a holding potential of −80 mV every 10 s with a bolus injection, or by voltage steps to different test potentials from a holding potential of −80 mV with the perfusion method. Figure 4*Aa* shows a representative response. The size of the subtraction current before and after injection of AII showed that AII substantially reduced I_A and partly reduced the delayed component (Fig. 4*Ab*). The decrease of peak outward current occurred within 30 s of the application of AII and recovered within 5 min (Fig. 4*B*). These responses were reversible and repeatable. The current (I_{peak})–voltage (V_{test}) relationship before and after perfusion of AII showed that AII did not affect the threshold of I_A but clearly diminished its amplitude (Fig. 4*C* and *D*). The decrease of I_A was calculated as the percentage change in peak outward current and expressed as percentage

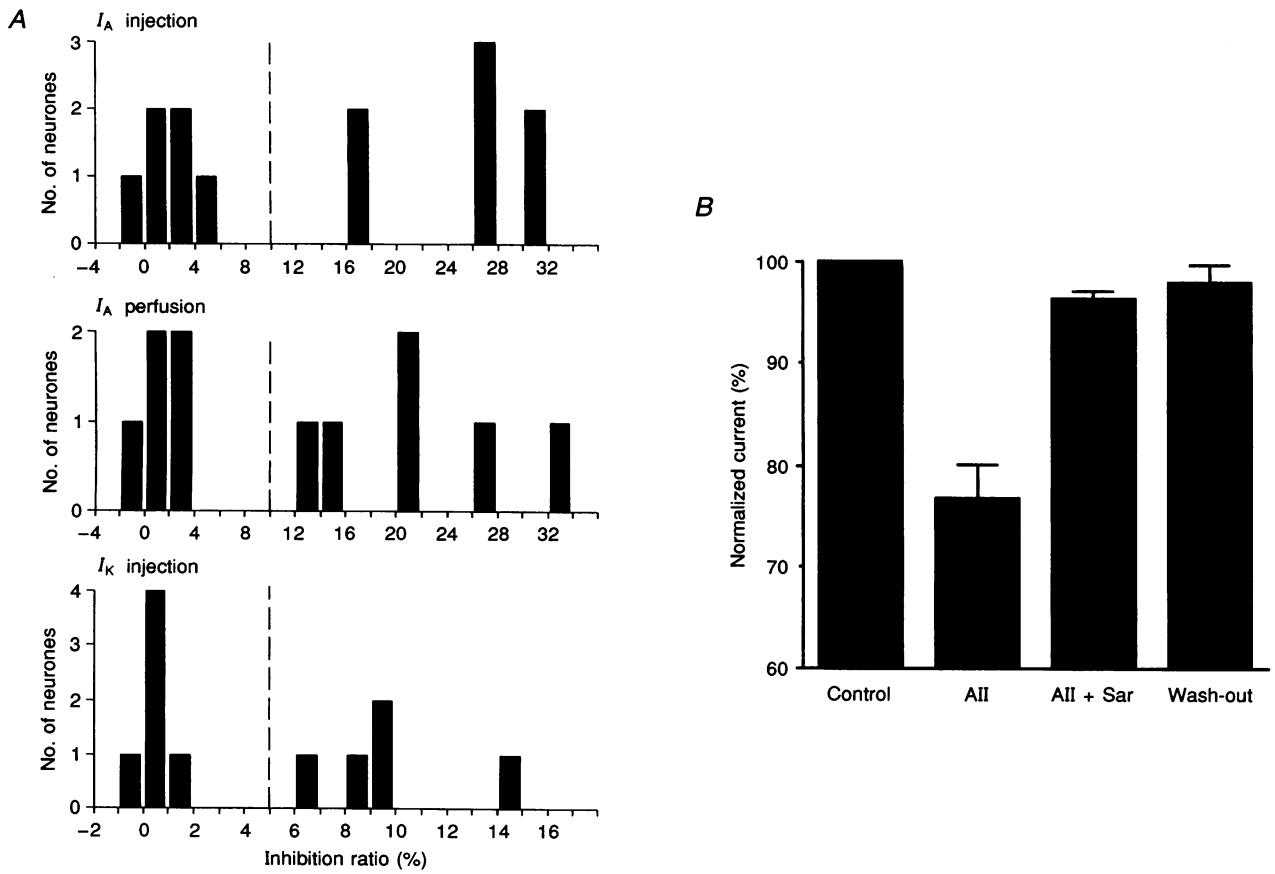

Figure 5. Histograms of the cell responses to AII and effects of saralasin on the AII-induced current

A, the histograms show the cell responses to AII using three experimental methods, the injection method for I_A (I_A injection), the perfusion method for I_A (I_A perfusion) and the injection method for I_K (I_K injection). The horizontal axis indicates the percentage inhibition compared with the control and the vertical axis indicates the number of neurones. The cells showing a decrease in amplitude of more than 10% in I_A and more than 5% in I_K were classified as sensitive. Dashed lines separate the two groups. The differences between sensitive and low-sensitive groups were statistically significant ($P < 0·05$). *B*, the effects of saralasin on AII-induced current in three SON neurones. The currents were normalized to the amplitude before AII application (control). Saralasin blocked the AII-induced suppressing effects (AII + Sar). Each bar represents the mean ± S.E.M.

inhibition in relation to the control. The mean values for the percentage reduction were 14·6 ± 3·5% ($n = 13$) by bolus injection of AII and 12·4 ± 3·5% ($n = 11$) by perfusion of AII.

We analysed the change of outward currents by AII in individual cells. Figure 5*A* shows the histograms of cell responses to AII. Judging from the distributions of cell responses, cells were arbitrarily classified into two groups, sensitive and low-sensitive, with the borderline at 10%. Bolus injection of AII decreased the I_A amplitude 25·1 ± 2·4% in seven (53·8%) of the thirteen neurones tested (sensitive group), whereas the other six neurones changed only by 2·2 ± 0·8% (low-sensitive group). Perfusion of AII decreased the I_A amplitude by 21·3 ± 3·2% at 0 mV in seven (54·5%) of the eleven neurones tested (sensitive group), whereas the other five neurones changed only by 1·7 ± 0·8% (low-sensitive group).

The effects of AII on delayed rectifier current (I_K) were also studied in eleven SON neurones. The membrane potential was stepped to +40 mV from a holding potential of −40 mV, and AII (10 μM, 100 μl) was applied by bolus injection. The AII response was evaluated at the end of a 300 ms voltage step. The mean value of inhibition ratio was 4·6 ± 1·6% ($n = 11$). Judging from the distribution of cell responses in Fig. 5*A*, cells were also arbitrarily classified into two groups, sensitive and low-sensitive, with a borderline at 5%. AII decreased I_K amplitude 9·6 ± 1·6% in five (45·5%) of the eleven neurones tested (sensitive group), whereas the other six neurones changed by only 0·46 ± 0·27% (low-sensitive group). The difference of inhibition ratio between I_A and I_K in the sensitive groups (I_A injection *vs.* I_K injection) was statistically significant ($P < 0·05$).

The effects of saralasin on AII-induced responses were tested in three neurones, the I_A of which had been suppressed by AII (Fig. 5*B*). Perfusion medium containing 1 μM saralasin was applied for 5 min before the application of AII and superimposed during the application of AII. In the three neurones, AII decreased the normalized peak amplitude of I_A by 76·8 ± 3·4%. The suppressing effects of AII were almost completely blocked by saralasin treatment and the amplitude of I_A was restored to the control value after wash-out. The normalized I_A values were 96·3 ± 0·7% with the saralasin treatment and 98·0 ± 1·6% after wash-out. These results provide evidence that the responses of SON neurones to AII is specific.

The effects of AII on the steady-state inactivation were investigated in four SON neurones (see Fig. 6*A*). The peak currents were decreased by AII application over the whole range of the pre-pulse potentials. Figure 6*B* shows the peak current normalized to the current which was obtained from a pre-pulse potential of −120 mV. The half-inactivation voltage ($V_{½}$) of the inactivation curve was shifted in this neurone by only −1·5 mV after perfusion of AII (0·1 μM). The other three neurones tested also showed little shift of the half-inactivation voltage. These results indicate that AII decreased I_A without changing the voltage dependence of steady-state inactivation.

DISCUSSION

The present study confirms the basic characteristics of I_A recorded from microscopically identified SON neurones in the rat hypothalamic slice preparation with the whole-cell patch-clamp technique and reveals the suppressive effect

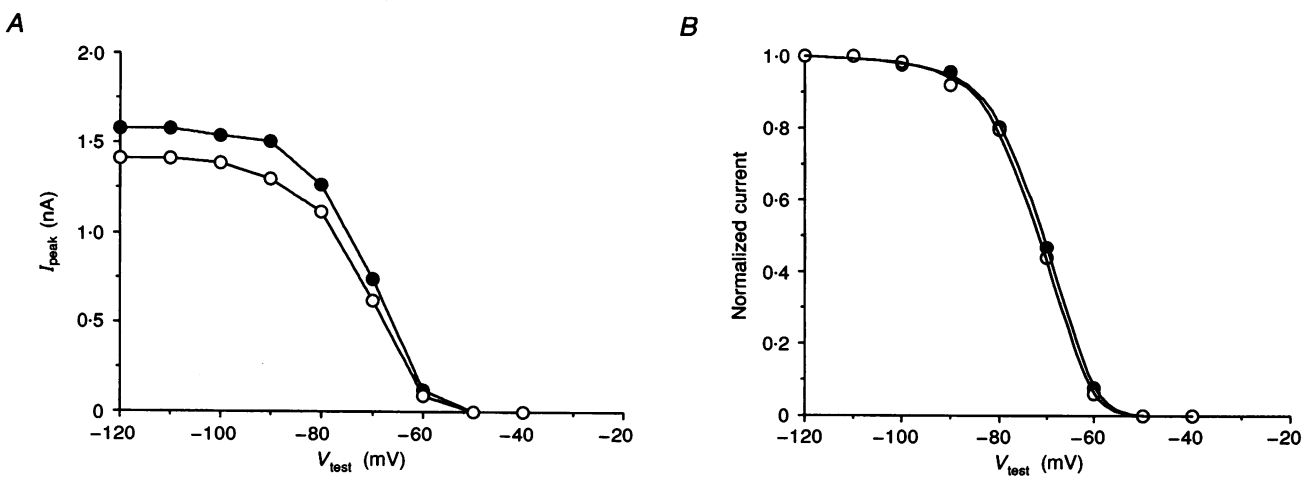

Figure 6. Effects of AII on steady-state inactivation of I_A

A, steady-state inactivation curves of peak currents before (●) and after (○) perfusion of AII (0·1 μM). The currents were measured at 0 mV from different holding potentials (V_{test}) in the range of −120 to −40 mV. *B*, the currents were normalized to the ratio of the largest currents before and after AII application. AII shifted the steady-state inactivation curve in the negative direction by only 1·5 mV.

of AII on the I_A. The existence of the I_A in SON neurones has already been reported in cultured cells dissociated from 2- to 4-day-old rats (Cobbett *et al.* 1989) and cells from 13- to 14-day-old embryos using the whole-cell patch-clamp technique (Müller *et al.* 1992), or in perfused explants using the single-microelectrode voltage-clamp technique in adult rats (Bourque, 1988).

The I_A of supraoptic neurones in slices activated and inactivated rapidly. It was also completely inactivated at −50 mV. The half-inactivation voltage of steady-state inactivation was −72·0 mV. It has been reported that the values of the inactivation time constants and their voltage dependence differs in different species (Rudy, 1988). A previous paper has shown a partial voltage dependence of the inactivation time constants of I_A in cultured neurones of the SON area (Cobbett *et al.* 1989). In our study, time constants for the inactivation of I_A obtained from the current decay ranged between 7 and 60 ms and were voltage dependent. In addition, I_A was not inactivated completely at any resting membrane potential which we tested. The I_A may thus contribute to modulation of neuronal activity by carrying a steady-state current around the resting membrane potential (Bourque, 1988) or contribute to repolarization of the action potential (Belluzzi, Sacchi & Wanke, 1985).

The reversal potential of I_A obtained from the double voltage-step protocol in our experiments was approximately −70 mV. The estimated potassium equilibrium potential from the Nernst equation is −83·9 mV. Values of the reversal potentials in cultured cells from the SON area and in SON neurones in perfused explants were −80 mV (Cobbett *et al.* 1989), and −97 mV (Bourque, 1988), respectively. Our estimate of the reversal potential value is thus higher than that reported earlier. This may be because we added 2 mM $CoCl_2$ to the perfusion medium to block Ca^{2+} currents. We did this because inward currents carried by Ca^{2+} were activated by the step pulses and these masked outward currents (T. Nagatomo, K. Inenaga & H. Yamashita, unpublished observation). It has been reported that the presence of Co^{2+} in the external solution shifts the activation and inactivation curves towards a more positive value by modulating the membrane surface charge (Numann, Wadman & Wong, 1987; Tsuda, Oyama, Carpenter & Akaike, 1988; Bourque, 1988). Thus, Co^{2+} in the perfusion medium might have shifted the reversal potential of I_A. Alternatively the use, in the perfused explant preparation, of electrodes filled with 3 M potassium acetate or chloride may have allowed sufficient leakage of the electrode solution into the cell to shift the potassium equilibrium potential. The reversal potential of I_A in our study was shifted 17·2 ± 0·25 mV ($n = 3$) by changing the potassium concentration in the perfusion medium from 5 to 10 mM (data not shown). This shift of the reversal potential is close to the value estimated from the Nernst equation (17·8 mV at 25 °C). Thus, the reversal potential in the present study was almost entirely determined by the movement of potassium ions.

AII has been suggested as a neurotransmitter or neuromodulator in the hypothalamus (Yamashita & Kannan, 1992). SON neurones can be divided into two groups on the basis of their firing pattern. We (Yamashita, Inenaga, Kawata & Sano, 1983) and others (Cobbett, Smithson & Hatton, 1986) have reported, by combining immunohistochemical and electrophysiological techniques, that most of the phasically firing neurones (phasic neurones) contain vasopressin while the other neurones which did not fire phasically (non-phasic neurones) contain oxytocin. We have already shown using extracellular recording that AII excites 44% of SON neurones tested and that there was no significant difference between the proportion of vasopressin neurones (phasic firing, 40%) and oxytocin neurones (non-phasic firing, 48%) which were excited by AII (Okuya *et al.* 1987). AII significantly inhibited I_A in about 50% of the SON neurones in the present study. The proportion of neurones which were sensitive to AII was thus similar to that in our previous study (Okuya *et al.* 1987). Although we did not identify the cell type electrophysiologically in the present study, it is likely that AII acted on both the oxytocin and the vasopressin neurones in the SON. Since one of the roles of I_A is to regulate firing frequency (Connor & Stevens, 1971), it is reasonable to speculate that inhibition of I_A by AII induces the excitation of both oxytocin and vasopressin neurones in the SON. Recently, it has been reported that AII depolarizes rat supraoptic neurones through a non-selective cationic channel by activating the type I receptor of AII (Yang, Phillips & Renaud, 1992). These two lines of evidence suggest that AII excites SON neurones both by suppression of I_A and by enhancement of non-selective cationic currents. Thus, AII may excite SON neurosecretory cells, at least in part, through suppression of I_A and in this way increase vasopressin and oxytocin release.

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