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TWO TRANSIENT OUTWARD CURRENTS IN HISTAMINE NEURONES OF THE RAT HYPOTHALAMUS *IN VITRO*

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SUMMARY

1. The transient outward current exhibited by the histamine neurones of the tuberomammillary nucleus was studied using the single-electrode voltage clamp technique in an *in vitro* rat hypothalamic slice preparation.

2. The transient outward current exhibited steady-state inactivation at the resting potential. Inactivation was removed by priming hyperpolarization with a $V_{\frac{1}{2}}$ of $-85 \pm 1{\cdot}2$ mV, while the $V_{\frac{1}{2}}$ for activation was $-60{\cdot}3 \pm 2{\cdot}1$ mV.

3. The decay of the transient outward current was best fitted by two exponentials with time constants of 104 ± 36 and 568 ± 128 ms. These two components were provisionally termed $I_{A,f}$ and $I_{A,s}$ for the fast and slowly decaying currents, respectively.

4. Removal of inactivation was time dependent; inactivation was fully removed by hyperpolarizing pulses to -110 mV of 200 ms or greater duration. Removal of inactivation of $I_{A,f}$ was rapid, becoming complete with hyperpolarizing pre-pulses of 50 ms or greater, while removal of inactivation of $I_{A,s}$ was not complete until hyperpolarizing pre-pulses were 200 ms in duration.

5. The fast decaying current $I_{A,f}$ was selectively blocked by 1 mM-4-aminopyridine. Tetraethylammonium chloride (10 mM) had no effect on either $I_{A,f}$ or $I_{A,s}$.

6. The inactivation curves for $I_{A,s}$, determined both by using the values obtained from the amplitude of the computed slower exponential function as well as that of the current remaining in 1 mM 4-aminopyridine, were negative to those of $I_{A,f}$. Similarly derived activation curves for $I_{A,s}$ were positive to those of $I_{A,f}$.

7. Superfusion with a nominal 0 Ca^{2+} medium containing 10 mM-Mg^{2+} did not reduce the maximal transient outward current.

8. The reversal potential of $I_{A,s}$ with 2·5 mM-K^+ in the medium was -95 ± 3 mV; the reversal potential of $I_{A,f}$ was at least 15 mV negative to that of $I_{A,s}$.

9. It is concluded that histaminergic tuberomammillary neurones possess at least two transient outward currents which can be distinguished on the basis of their rates of decay, 4-aminopyridine sensitivity, voltage dependence and reversal potentials.

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INTRODUCTION

The existence of transient outward K^+ currents (I_A) in neuronal somata was first described by Connor & Stevens (1971*a*) and Neher (1971). Since the initial reports, I_A has been found in a wide variety of excitable tissues (Rogawski, 1985; Rudy, 1988). The hallmarks of this current are its steady-state inactivation near the resting potential, removal of inactivation with hyperpolarization, activation upon subsequent depolarization, and exponential decay.

The role of I_A in modifying excitability varies in different cells, reflecting such factors as the spatial distribution of A-type K^+ channels, and the voltage- and time-dependence of I_A activation and inactivation. Moreover, while many cells exhibit one or more currents described as I_A, considerable heterogeneity has been reported. While most transient outward currents are sensitive to blockade by aminopyridines, others appear to be resistant (Adams, Brown & Constanti, 1982; Salkoff, 1983; Zbicz & Weight, 1985; Lukasiewicz & Werblin, 1988). The time course of decay of I_A varies in different cells, and transient outward currents with different decay rates can be recorded within the same cell (Coraboeuf & Carmeliet, 1982; Zbicz & Weight, 1985; Hiraoka & Kawano, 1988; Lukasiewicz & Werblin, 1988; Storm, 1988). Moreover, molecular studies have shown that multiple genes code for I_A in *Drosophila* (Salkoff, 1983; Solc, Zagotta & Aldrich, 1987; Butler, Wei, Baker & Salkoff, 1989), and that alternative mRNA splicing results in ion channels with different inactivation kinetics (Timpe, Schwarz, Tempel, Papazian, Jan & Jan, 1988).

In a recent study of the membrane properties of histaminergic tuberomammillary neurones of the rat hypothalamus, it was observed that these neurones exhibit remarkably long-lasting transient outward rectification (Haas & Reiner, 1988). We have now studied this phenomenon using the single-electrode voltage clamp technique, and the results indicate that tuberomammillary neurones possess at least two distinct transient outward currents. A preliminary report of these studies has appeared in abstract form (Reiner, Greene & Haas, 1989).

METHODS

Slices of the mammillary body were prepared as previously described (Reiner & McGeer, 1988). Briefly, young rats of 50–150 g were anaesthetized with ether, decapitated, and the brain rapidly removed and immersed in cold artificial cerebrospinal fluid. The hypothalamus was dissected free, and coronal slices of 300 μm thickness were cut on a vibratome. Slices were stored in a holding chamber for at least 1 h prior to recording. The recording chamber was a modification of a previous design (Haas, Schaerer & Vosmansky, 1979) in which slices were continuously superfused with warmed (30 °C) artificial cerebrospinal fluid at a flow rate of 1·5–2 ml min^{-1}. In order to reduce electrode capacitance, the fluid level was reduced to within 400 μm of the upper surface of the slice; under these conditions, total chamber volume was $\sim$ 250 μl. The tuberomammillary region was readily identified at the extreme lateral and ventral edge of the brain at the level of the mammillary recess; all recordings were obtained from neurones in the lateral and ventral subnuclei of the tuberomammillary nucleus in which histaminergic tuberomammillary neurones are most densely concentrated (Reiner, Semba, Fibiger & McGeer, 1988).

Intracellular recordings were carried out using 1·2 or 1·5 mm o.d. micropipettes filled with 2 M-KCl with resistances ranging between 25 and 60 MΩ connected to an Axoclamp 2A amplifier. Data were acquired using the PCLAMP suite of programs and an Axolab 1100 interface, which also served to generate current and voltage commands. Data were also independently digitized at 49 kHz and stored on videotape for off-line analysis. Tuberomammillary neurones reported in this study had

membrane properties essentially identical with those previously reported for immunohistochemically identified histamine neurones (Haas & Reiner, 1988), including membrane input resistances of 150–400 MΩ, action potentials of 1·8–2·2 ms duration arising from a threshold of −53 mV, and both inward and transient outward rectification. Following characterization in bridge mode, tetrodotoxin (0·1 μM) was applied and the amplifier switched into single-electrode voltage clamp mode. Switching frequencies ranged from 4 to 8 kHz, with gains of 0·5–1 nA mV^{-1}, and the signal was filtered at 1 kHz. All traces in single-electrode voltage clamp mode are the average of four trials. Leakage and capacitive currents were not subtracted. Headstage output was continuously monitored on an independent oscilloscope to ensure that the voltage at the electrode had settled prior to the sample-and-hold measurement. Settling time of the clamp for a 40 mV step was 2–5 ms. As has been extensively discussed in the literature, single-electrode voltage clamp suffers from the limitations of point clamping, and thus the data obtained with this technique are necessarily treated as qualitative (Johnston & Brown, 1983).

The composition of the artificial cerebrospinal fluid was (in mM): Na^+, 152; K^+, 2·5; Ca^{2+}, 2·4; Cl^-, 136; PO_4^{2-}, 1·2; Mg^{2+}, 1·3; CO_3^-, 25; glucose, 11; pH 7·4 when saturated with 95% O_2, 5% CO_2. The nominal 0 Ca^{2+} medium was identical except that Ca^{2+} was omitted and the Mg^{2+} concentration was raised to 10 mM. The following drugs (all obtained from Sigma) were bath applied in concentrations detailed in the text: 4-aminopyridine (4-AP), tetraethylammonium chloride (TEA), and tetrodotoxin. Addition of 1 mM-4-AP caused the artificial cerebrospinal fluid to become basic, and therefore such solutions were titrated to pH 7·4 with 1 M-HCl. Data are reported as mean ± standard deviation. Activation and inactivation curves were fitted using the curve-fitting feature of Harvard Graphics (Software Publishing Co.).

RESULTS

The transient outward current was studied in fifty-three tuberomammillary neurones. In bridge mode, transient outward rectification was observed upon the offset of current pulses which produced sufficiently large membrane hyperpolarizations from rest; when the same neurone was studied under voltage clamp, the offset of similar hyperpolarizing voltage steps elicited a transient outward current (Fig. 1). Both activation and inactivation were well fitted ($R = 0{\cdot}98 \pm 0{\cdot}01$) by a function of the form

$$I/I_{max} = [1 + \exp(V - V_{\frac{1}{2}})/k]^{-1}, \quad (1)$$

where I_{max} is the maximum transient outward current, I is the transient outward current evoked in response to a command potential of V, $V_{\frac{1}{2}}$ the potential at which the current is half-activated and k is a slope factor describing the steepness of activation or removal of inactivation. A logarithmic transform of eqn (1) was fitted by the least-squares method to obtain values for $V_{\frac{1}{2}}$ and k in eight cells. The value of $V_{\frac{1}{2}}$ for activation was $-60{\cdot}3 \pm 2{\cdot}1$ mV with a slope factor of $5{\cdot}9 \pm 1{\cdot}4$ mV, while $V_{\frac{1}{2}}$ for inactivation was $-88{\cdot}5 \pm 3{\cdot}3$ mV and the slope factor was $-6{\cdot}0 \pm 0{\cdot}7$ mV. For all fitted functions the correlation coefficient, R was $\geqslant 0{\cdot}98$.

The decay of the transient outward current following a 400 ms step to −110 mV from a holding potential of −50 mV was best fitted by a double-exponential function of the form

$$I = A_0 + A_f \exp(-t/\tau_f) + A_s \exp(-t/\tau_s), \quad (2)$$

where I is the peak transient outward current, A_0 the steady-state current at time zero, A_f and A_s the maximal amplitudes of the fast and slowly decaying components, respectively, t is time and τ_f and τ_s the time constants of decay for the fast and

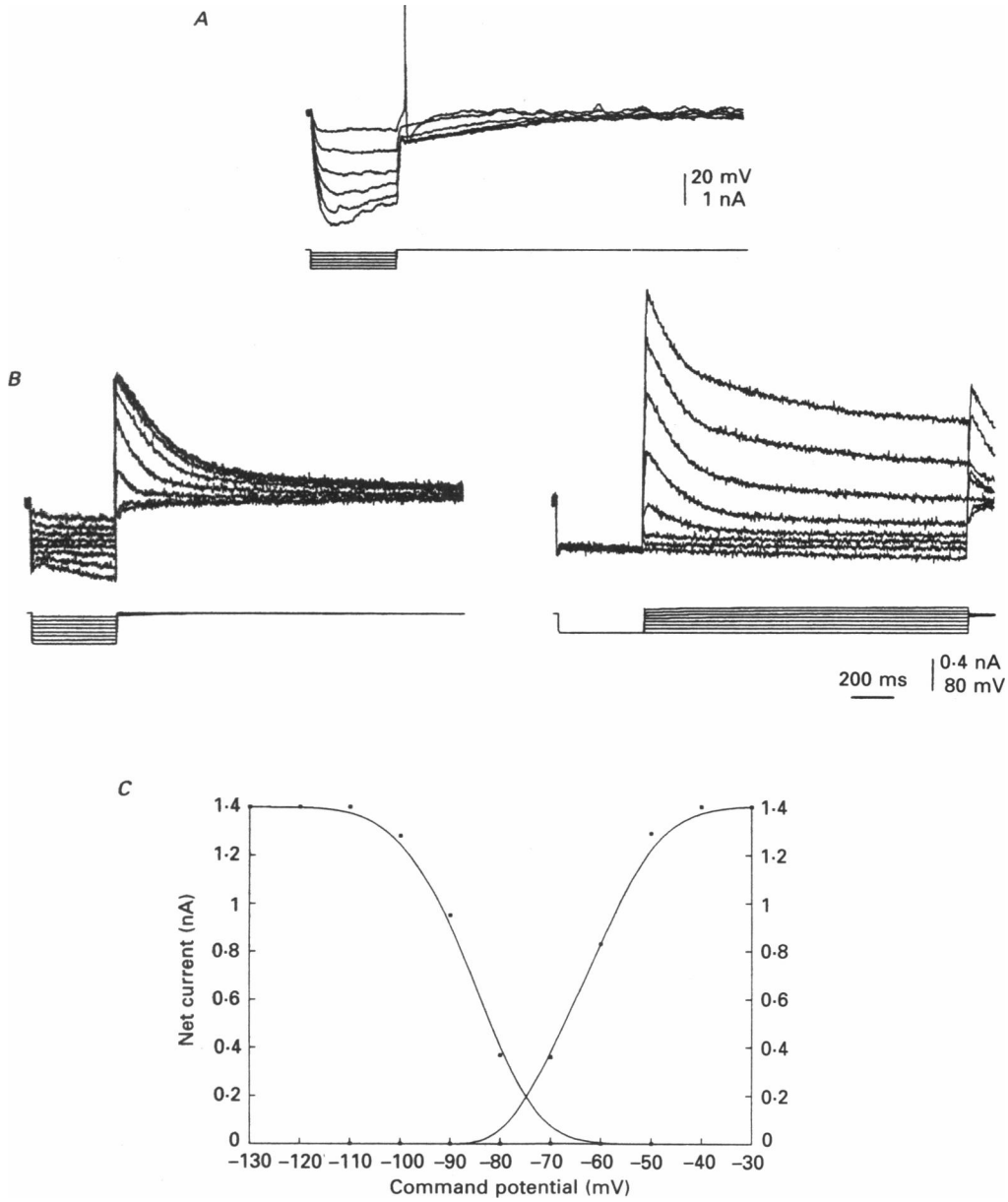

Fig. 1. Activation and inactivation of I_A. *A*, in bridge mode, a family of hyperpolarizing current pulses from a holding potential of −60 mV evokes transient outward rectification. *B*, in single-electrode voltage clamp mode, hyperpolarizing voltage steps from a holding potential of −50 mV remove steady-state inactivation of I_A which is evoked upon depolarization. *C*, activation and inactivation curves of I_A for the cell shown above. Net current was measured by subtracting the peak transient outward current from the steady-state current measured at the end of the voltage step.

slowly decaying currents, respectively. For eighteen cells, τ_f was 104 ± 36 and τ_s was 568 ± 128 ms (Fig. 2*B*). While double-exponential inactivation of the transient outward current is not in and of itself compelling evidence for the existence of two distinct currents, data presented below suggest that this is indeed the case, and we have therefore operationally defined two components of the transient outward current in tuberomammillary neurones as $I_{A,f}$ and $I_{A,s}$ for the fast and slowly decaying currents, respectively.

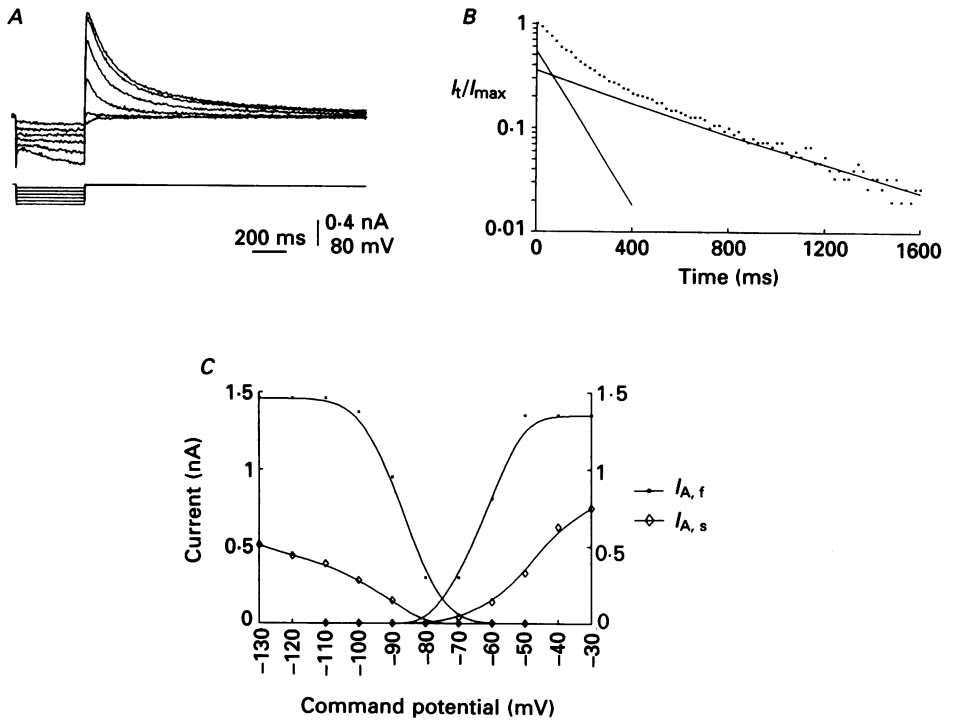

Fig. 2. The decay of I_A is best fitted by two exponential functions. *A*, the current resulting from a family of hyperpolarizing voltage steps (−110 to −60 mV) from a holding potential of −50 mV is shown. *B*, normalized current (I_t/I_{max}) following a hyperpolarizing pre-pulse to −110 mV is plotted against time as a dotted line on a semilogarithmic scale. The two exponential functions representing time constants of 121 and 550 ms are superimposed on this plot as continuous lines. *C*, activation and inactivation curves of $I_{A,f}$ (dots) and $I_{A,s}$ (open diamonds). The graph was generated by plotting the values of A_f and A_s obtained from eqn (2) (see text) *vs.* the command potential.

The amplitude of the fast transient outward current (A_f) invariably dominated the peak current, comprising $66{\cdot}5 \pm 8{\cdot}1$% of the total transient outward current *vs.* $33{\cdot}5 \pm 7{\cdot}2$% for A_s. When A_f and A_s are plotted with respect to the command potential, $I_{A,s}$ appears to activate at potentials considerably positive to $I_{A,f}$ and removal of inactivation of $I_{A,s}$ occurs negative to that of $I_{A,f}$ (Fig. 2*C*). Moreover, at the maximal potentials for which adequate voltage control could be obtained, $I_{A,s}$ did not appear to reach its maximal value.

The time dependence of removal of inactivation was studied in five neurones by holding the membrane at −50 mV and stepping to −110 mV for times ranging from 12·5 to 400 ms (Fig. 3). In all cells examined, hyperpolarizing pre-pulses of greater

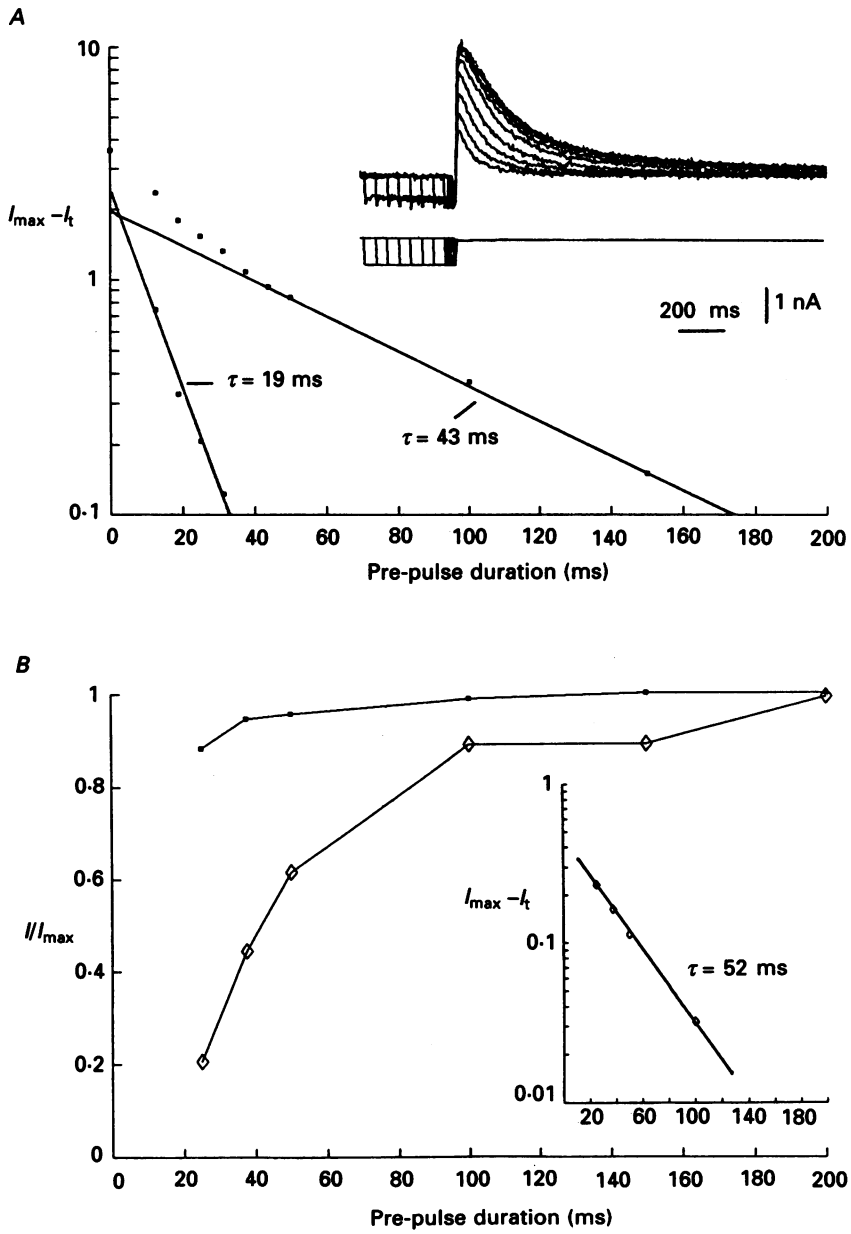

Fig. 3. Time dependence of removal of inactivation. *A*, the membrane was held at −50 mV, and stepped to −110 mV for variable periods of time from 400 to 12·5 ms. In each case, the timing of the start of the hyperpolarizing voltage step was incremented while the offset of the hyperpolarizing voltage steps was synchronized. The trace in the inset shows a subset of the total number of test pulses. In all cells tested, hyperpolarizing voltage steps of greater than 200 ms fully removed inactivation. The maximal transient outward current evoked by a hyperpolarizing pulse of 400 ms (I_{max}) minus the peak current measured following hyperpolarizing pre-pulses of various times (I_t) is plotted on

than 200 ms fully removed inactivation. The peak outward current measured upon return to the holding potential following hyperpolarizing pre-pulses of various durations (I_t) was subtracted from the peak outward current elicited by 400 ms duration hyperpolarizing pre-pulses (I_{max}), and this quantity was plotted with respect to the duration of the hyperpolarizing pre-pulse. When examined in this fashion, the time dependence of removal of inactivation could be fitted by a double-exponential function with time constants of $17{\cdot}5 \pm 3{\cdot}2$ and $47{\cdot}1 \pm 5$ ms (Fig. 3*A*). In two cells, the amplitudes of A_f and A_s obtained with eqn (2) were plotted against the duration of the hyperpolarizing pre-pulse. Such analysis revealed that removal of inactivation of A_s required longer pre-pulses than that required by A_f (Fig. 3*B*). Moreover, the time constants of removal of inactivation of A_s were 48 and 52 ms, similar to the mean slower time constant of removal of inactivation of peak transient outward current obtained using the analysis shown in Fig. 3*A*. The limitations of the single-electrode voltage clamp with very short pulse durations precluded similar analysis of A_f whose amplitude did not begin to diminish until hyperpolarizing pre-pulses were 25 ms or less in duration (Fig. 3*B*). None the less, these data suggest that $I_{A,f}$ and $I_{A,s}$ exhibit different time dependences for removal of inactivation.

Effects of K^+ channel blockers

Tetraethylammonium had no significant effect on either $I_{A,f}$ or $I_{A,s}$ ($n = 5$, Fig. 4*A* and *B*). As predicted on the basis of current clamp data (Haas & Reiner, 1988), TEA potently reduced the delayed outward current(s) evoked by depolarization from -50 mV (Fig. 4*C*).

The fast decaying transient outward current ($I_{A,f}$) was blocked by 1 mM-4-AP, while $I_{A,s}$ was unaffected (Fig. 5*A*, *B* and *D*). As previously described (Thompson, 1982), 4-AP blockade of $I_{A,f}$ exhibited voltage dependence; when the membrane was hyperpolarized for 1 min beyond -100 mV, depolarization to -50 mV revealed complete blockade of $I_{A,f}$. When the holding potential was subsequently changed to -50 mV, $I_{A,f}$ returned to $17 \pm 3\%$ of control upon the offset of a 200 ms duration hyperpolarizing command to -110 mV ($n = 4$, Fig. 5*C*). The decay of the current persisting in 1 mM-4-AP was well fitted by a single-exponential function, and its time constant was the same as that of $I_{A,s}$ in control solutions (Fig. 5*D*). The slowly decaying current ($I_{A,s}$) appears to be the voltage clamp analogue of the slow component of transient outward rectification seen in bridge mode since it persisted in the presence of 1 mM-4-AP (inset, Fig. 5*B*).

Because $I_{A,f}$ comprises 66% of the peak transient outward current in control solutions and $I_{A,s}$ comprises at least 89% of the peak current in 1 mM-4-AP (at a

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a semilogarithmic scale against the duration of the pre-pulse. The longer pulses resolve to a straight line (fitted by eye) with a time constant of 43 ms. Extrapolating this line to zero and peeling results in a second exponential function with a time constant of 19 ms. *B*, the transient outward current evoked by hyperpolarizing pre-pulses of different durations was fitted using eqn (2), and normalized values (I/I_{max}) for A_f (■) and A_s (◇) were plotted against the pre-pulse duration. A_s requires longer pre-pulses to remove inactivation than A_f. Inset: semilogarithmic plot of $I_{max} - I_t$ *vs.* pre-pulse duration for A_s resolves to a single exponential with a time constant of 52 ms.

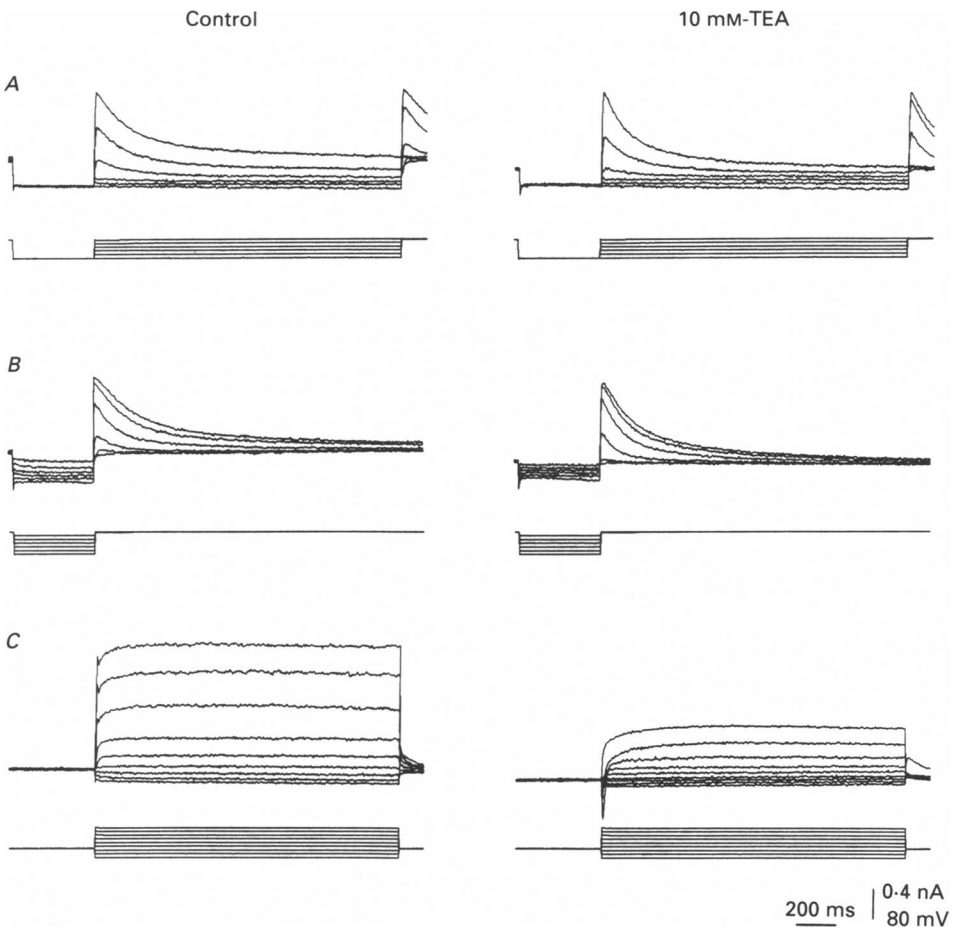
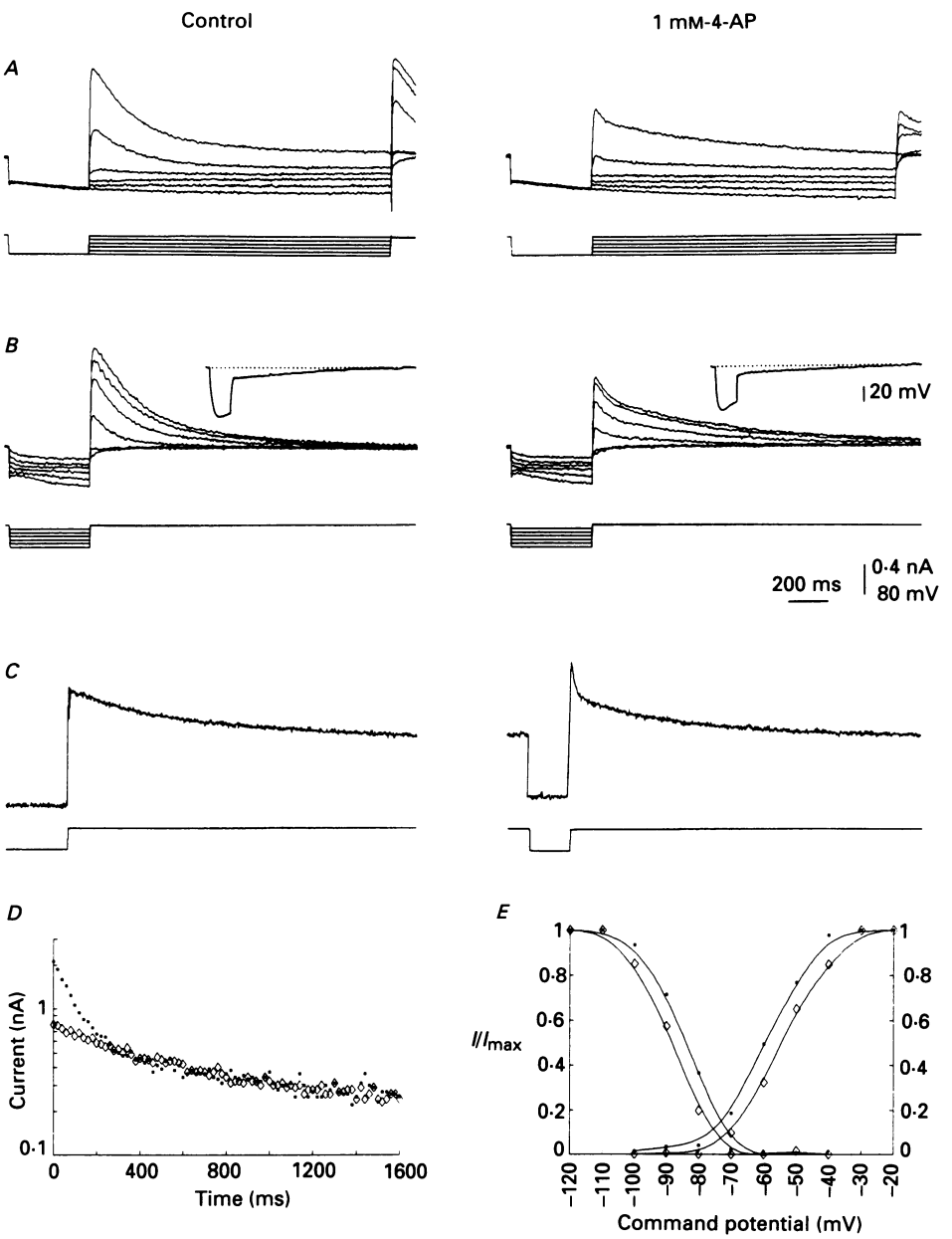


Fig. 4. Effects of TEA on I_A. *A* and *B*, 10 mM-TEA causes a small inward current shift and reduces the steady-state current at the end of the step, but has no effect on either $I_{A,f}$ or $I_{A,s}$. Holding potential = −50 mV. *C*, in the same cell, TEA markedly reduces the non-inactivating outward current(s) evoked by depolarizing voltage steps from −50 mV.

holding potential of −50 mV), this pharmacological manipulation can be used to obtain a qualitative estimate of the activation and removal of inactivation of $I_{A,f}$ and $I_{A,s}$. The inactivation curve was shifted in the negative direction by 5–10 mV by 4-AP treatment, with half-maximal inactivation occurring at -92 ± 4 mV ($n = 4$).

Fig. 5. Effects of 4-AP on I_A. *A* and *B*, at a holding potential of −50 mV, 1 mM-4-AP markedly reduces $I_{A,f}$, leaving $I_{A,s}$ intact. Inset: the long-lasting transient outward rectification seen in bridge mode is slightly reduced in amplitude but its time course is unaffected by treatment with 4-AP. *C*, 4-AP blockade of $I_{A,f}$ is voltage dependent. On the left, the membrane potential was held at −110 mV for 1 min and 2 s depolarizing pulses to −50 mV were applied with a 5 s interpulse interval in the presence of 1 mM-4-AP (trace shown is the average of four trials). Under these conditions, $I_{A,f}$ is completely blocked. Subsequently, the holding potential was changed to −50 mV for 1 min, and the paradigm



repeated with 200 ms hyperpolarizing pulses to −110 mV. Blockade of $I_{A,f}$ by 1 mM-4-AP is incomplete at −50 mV. *D*, superimposition of the transient outward current evoked by a step from −110 mV to −50 mV from a cell under control conditions and following 1 mM-4-AP plotted on a semilogarithmic scale. Note that in the presence of 4-AP, I_A resolves to a single-exponential function which superimposes the slower portion of the current evoked under control conditions. *E*, normalized (I/I_{max}) activation and inactivation curves for peak I_A in control (dots) and 4-AP-containing medium (diamonds) show that 4-AP shifts the inactivation curve approximately 5 mV in the negative direction, while producing a similar sized positive shift in the activation curve.

Simultaneously, the activation curve was shifted in the positive direction some 5–10 mV by 4-AP treatment (Fig. 5*E*).

Effects of Ca^{2+} removal and channel blockade

When tuberomammillary neurones were perfused with artificial cerebrospinal fluid in which Ca^{2+} was omitted and the Mg^{2+} concentration raised to 10 mM, there was no

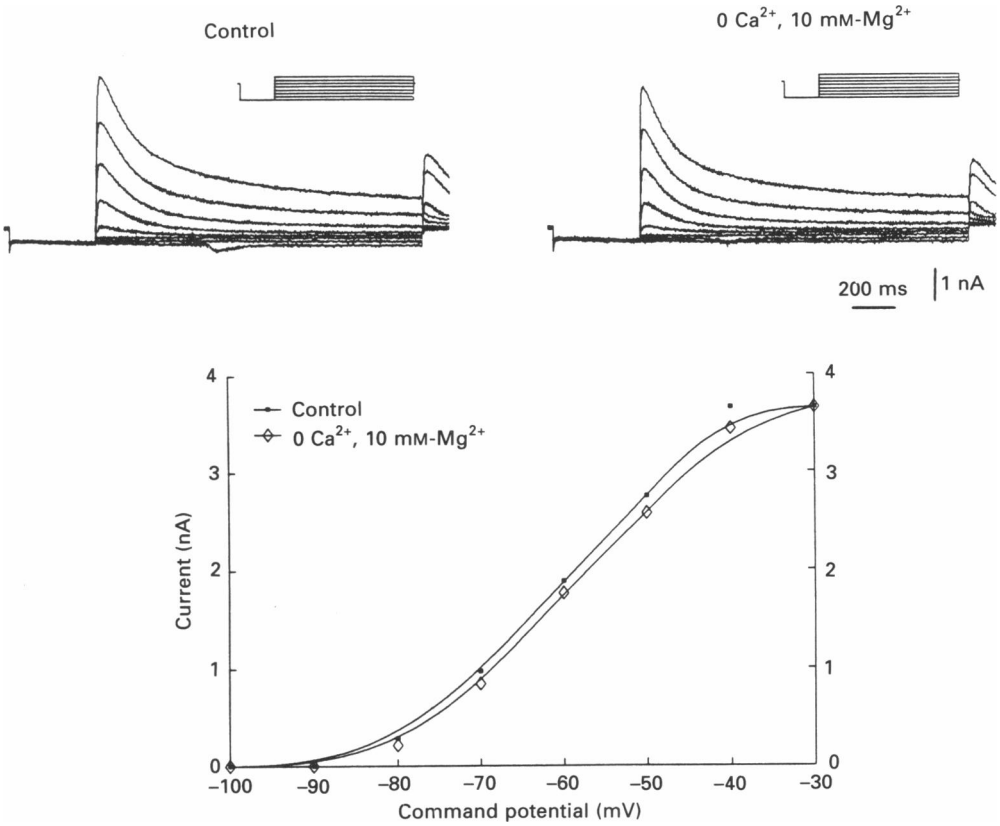


Fig. 6. Effects of Ca^{2+} removal on activation of I_A. At the top are shown the currents evoked by hyperpolarization to −100 mV for 400 ms, followed by a 1·6 s depolarizing pulse to potentials ranging from −100 to −30 mV in both control solutions and those in which Ca^{2+} was removed and the Mg^{2+} concentration raised to 10 mM. Holding potential was −50 mV. The maximal amplitude of the transient outward current was not affected by this treatment, although the activation curve (below) was shifted slightly to the right.

change in the maximal transient outward current nor in $I_{A,f}$ or $I_{A,s}$. This treatment did produce a parallel shift in the activation curve, with $V_{\frac{1}{2}}$ increasing from $-62 \pm 1{\cdot}4$ to $-59{\cdot}1 \pm 2{\cdot}4$ mV ($n = 4$, Fig. 6). This shift is most likely due to a surface charge effect secondary to the change in divalent cation concentrations (Frankenhauser & Hodgkin, 1957). Consistent with such an explanation, the peak transient outward current evoked using the protocol for studying removal of inactivation (400 ms hyperpolarizing pulses from a holding potential of −50 mV) was reduced by

$12 \pm 3{\cdot}4\%$ by changing to the 0 Ca^{2+}, 10 mM-Mg^{2+} medium. These data suggest that the transient outward currents of histamine neurones are not Ca^{2+} dependent.

Reversal potential of the transient outward current

The reversal potential of the transient outward current was studied by tail current analysis using a three-step protocol. Two experimental protocols were used. In one,

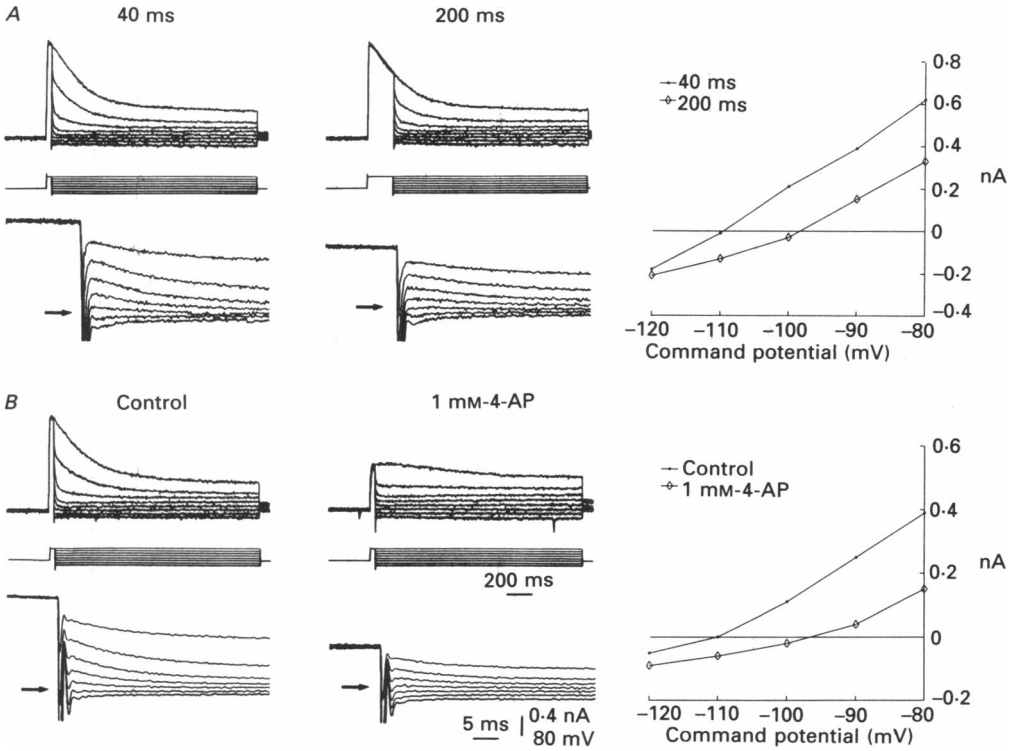


Fig. 7. Reversal potential of I_A. *A*, reversal potential of I_A was studied using a three-step protocol. The holding potential was −100 mV, with a step to −50 mV of either 40 or 200 ms duration, followed by test pulses to various potentials between −60 and −120 mV. The family of tail currents evoked by the third step is shown at higher sweep rate at the bottom of each trace with an arrow at the reversal potential, and the *I–V* curve for the tail currents are shown on the right. Data for the *I–V* curves were generated by measuring the current at the end of the 1·6 s tail current step and subtracting that value from the current measured 5 ms after the step. For the most hyperpolarized steps, the measurements were corrected for the inward rectification which develops during the 1·6 s step. At 40 ms, the current reverses at −110 mV, while at 200 ms, the current reverses at −98 mV. *B*, in another cell, the reversal potential of I_A was measured at 40 ms in the absence and presence of 1 mM-4-AP. The reversal potential for the control is −110 mV, while in 1 mM-AP, the current reverses at −96 mV.

the membrane potential was held at −100 mV, and depolarized to −50 mV for either 40 or 200 ms, followed by test pulses to various potentials. Based upon their exponential decays, at 40 ms $I_{A,f}$ and $I_{A,s}$ should contribute 66% and 34%, respectively, to the total the transient outward current, while at 200 ms $I_{A,f}$ accounts

for 29% and $I_{A,s}$ for 71%. Thus, the reversal potential of the tail current at 40 and 200 ms can be used to estimate qualitatively the reversal potentials of $I_{A,f}$ and $I_{A,s}$. When this protocol was applied in artificial cerebrospinal fluid containing 2·5 mM-K^+, the tail current measured at 40 ms reversed at -110 ± 2 mV ($n = 5$), while that at 200 ms reversed at -102 ± 4 mV (Fig. 7*A*). While these data suggest that $I_{A,f}$ and $I_{A,s}$ have different reversal potentials, the experiment suffers from the possibility that 200 ms depolarizing pulses might evoke a slowly developing inward current which summates with the outward current, or that sufficient K^+ has accumulated in the extracellular space during the 200 ms depolarizing pulse to alter the K^+ equilibrium potential. Since $I_{A,f}$ is fully blocked in the presence of 1 mM-4-AP at a holding potential of -100 mV, the reversal potential of $I_{A,s}$ at 40 ms can be examined in isolation. When such experiments were carried out, the reversal potential of the transient outward current which persisted in the presence of 4-AP was found to be -95 ± 3 mV ($n = 3$, Fig. 7*B*).

DISCUSSION

The co-existence of two distinct transient outward current components within individual cells has been reported in a variety of tissues, including mammalian heart (Coraboeuf & Carmeliet, 1982; Hiraoka & Kawano, 1988), *Drosophila* flight muscle (Salkoff, 1983), salamander retina (Lukasiewicz & Werblin, 1988) and rat hippocampal pyramidal neurones (Zbicz & Weight, 1985; Storm, 1988). The strongest support comes from the elegant studies of Salkoff (1983) in which it was shown that one of the two transient outward current components is absent in certain *Shaker* mutants.

Four lines of evidence suggest that tuberomammillary neurones possess two transient outward currents. First, when studied in voltage clamp, the transient outward current decays with double exponential kinetics. Secondly, only $I_{A,f}$ is sensitive to blockade by 4-AP. Thirdly, the activation and inactivation curves of $I_{A,f}$ and $I_{A,s}$ are different, whether examined using the amplitudes derived from the two exponentials which contribute to I_A or by comparing the curves obtained in the presence and absence of 4-AP. In addition, the kinetics of removal of inactivation differ for the two currents; $I_{A,s}$ requires longer hyperpolarizing pre-pulses to remove inactivation than does $I_{A,f}$. Fourthly, the reversal potential of $I_{A,s}$ tail currents (as measured in 1 mM-4-AP) is 15 mV depolarized to that of total I_A. Because $I_{A,s}$ contributes 33% to peak I_A in normal solutions, it was impossible to obtain a precise value for the reversal potential of $I_{A,f}$. None the less, the fact that $I_{A,s}$ exhibits a reversal potential different from that of total I_A suggests that $I_{A,f}$ and $I_{A,s}$ have different reversal potentials. Taken together, these data suggest that $I_{A,f}$ and $I_{A,s}$ are two distinct transient outward currents.

From a functional point of view, the most important difference between $I_{A,f}$ and $I_{A,s}$ is the time course of inactivation. The molecular basis for such differences is becoming clearer with the cloning of the genes which code for I_A in *Drosophila*. Multiple genes code for A-type K^+ channels in *Drosophila* (Salkoff, 1983; Solc *et al.* 1987; Butler *et al.* 1989). In addition, alternative mRNA splicing results in multiple

protein products from the *Shaker* gene (Schwarz, Tempel, Papazian, Jan & Jan, 1988), giving rise to at least two ion channels with different inactivation kinetics (Timpe *et al.* 1988). Moreover, in *Xenopus* oocytes injected with brain mRNA, inactivation of both Na^+ and K^+ channels is modified by a protein coded for by an mRNA species distinct from that which codes for the ion channel itself (Krafte, Snutch, Leonard, Davidson & Lester, 1988; Rudy, Hoger, Lester & Davidson, 1988). Thus, there exist at least three plausible mechanisms for generating A-currents with different inactivation kinetics.

A-currents have been suggested to play a role in controlling spike frequency, action potential repolarization and synaptic transmission (Connor & Stevens, 1971*b*; Jan, Jan & Dennis, 1977; Tanouye, Feerus & Fukita, 1981). A consistent feature of $I_{A,f}$ is that activation begins around -80 mV, while inactivation is not complete until the membrane has been depolarized beyond -60 mV. Threshold for action potential generation in histamine neurones is -53 mV and the after-hyperpolarization takes the membrane to the region of -75 to -80 mV (Haas & Reiner, 1988). This is precisely the region in which the activation–inactivation curves of $I_{A,f}$ overlap. Thus, a small percentage of $I_{A,f}$ should be evoked following each action potential and may contribute to the early portion of the interspike interval of histamine neurones. Given a time constant of inactivation of ~ 100 ms and 100% inactivation at potentials positive to -60 mV, very few $I_{A,f}$ channels would be expected to be open following a 500 ms interspike interval and it is therefore unlikely that $I_{A,f}$ plays a role in action potential repolarization.

While the time constant of inactivation of $I_{A,s}$ is approximately 500 ms, removal of inactivation of $I_{A,s}$ requires hyperpolarizations that are both greater in magnitude and longer in duration than $I_{A,f}$. It is therefore unlikely that sufficient $I_{A,s}$ is evoked during the after-hyperpolarization to contribute either to the interspike interval or to action potential repolarization. What role might $I_{A,s}$ play in histamine neurones? Hypothalamic neurones with anatomical and physiological properties consistent with their being histamine neurones have been reported to exhibit stereotypical changes in firing rate across the sleep–wake cycle in behaving animals, falling silent during rapid eye movement (REM) sleep (Vanni-Mercier, Sakai & Jouvet, 1984). This behavioral neurophysiological profile is strikingly similar to that exhibited by noradrenergic locus ceruleus neurones and serotonergic dorsal raphe neurones (Jacobs, 1987). The mechanism responsible for the state-related changes in firing rate of amine neurones is unknown, but it is widely believed that they are hyperpolarized during REM sleep. It is perhaps more than a coincidence that all three types of aminergic neurones exhibit long-lasting transient outward currents (Williams, North, Shefner, Nishi & Egan, 1984; Aghajanian, 1985). Thus one function of the slow decay of $I_{A,s}$ in amine neurones might be to ensure that the membrane potential remains in the region negative to threshold during REM sleep.

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