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CYCLIC AMP MEDIATES INHIBITION OF THE Na^+–K^+ ELECTROGENIC PUMP BY SEROTONIN IN TACTILE SENSORY NEURONES OF THE LEECH

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SUMMARY

1. Serotonin (5-HT) reduced the after-hyperpolarization (AHP) amplitude in tactile sensory neurones (T) but not in pressor (P) or nociceptive (N) cells of the leech.

2. Adenylate cyclase activators, phosphodiesterase inhibitors and membrane permeant analogues of cyclic adenosine monophosphate (cyclic AMP) mimicked the effect of 5-HT in reducing the AHP amplitude in T neurones.

3. Ionophoretic injection of cyclic AMP in T cells reduced the AHP amplitude, while cyclic guanosine monophosphate (cyclic GMP) or adenosine-5′-monophosphate (AMP) were without effect.

4. Inhibition of adenylate cyclase by the drug RMI 12330A (also known as MDL 12330A) suggested that 5-HT reduced the AHP amplitude through cyclic AMP.

5. 8-Bromoadenosine-3′-5′-cyclic monophosphate (8-Br-cyclic AMP) was still able to reduce the AHP amplitude after blocking the Ca^{2+}-activated K^+ conductance with $CdCl_2$ and converted the normal hyperpolarization which follows the intracellular injection of Na^+ into a depolarization. In addition, the cyclic AMP analogue slowed down and reduced the repolarization usually induced by CsCl after perfusion with K^+-free solution.

It is proposed that, in T sensory neurones, cyclic AMP mediates the inhibition of the Na^+–K^+ electrogenic pump induced by 5-HT application.

INTRODUCTION

In T mechanosensory neurones of the leech, a sustained discharge of action potentials induced either by intracellular electrical stimulation or by activation of their receptive fields, leads to an after-hyperpolarization (AHP). This phenomenon plays pivotal physiological roles including inhibition of the previously activated pathway, reduction of transmitter release, an enhancement of the volley threshold and conduction block at 'branching points' where the small neurites converge into the main axon (Baylor & Nicholls, 1969; Jansen & Nicholls, 1973; Yau, 1976). The AHP in T sensory cells is due mainly to the activation of the electrogenic Na^+–K^+ pump and partly to a Ca^{2+}-dependent K^+ conductance ($g_{K,Ca}$) (Baylor & Nicholls, 1969; Jansen & Nicholls, 1973; Van Essen, 1973).

It has been found that serotonin (5-HT) perfusion or intracellular electrical stimulation of the serotonergic Retzius cells leads to a reversible reduction of the AHP amplitude in T neurones, without altering either the input resistance or the resting potential (Belardetti, Brunelli, Demontis & Sonetti, 1984).

Recent experiments have analysed the mechanism through which 5-HT might act. By testing the effect of 5-HT on the residual AHP after inhibiting $g_{K,Ca}$ or on T cells in which the activity of the Na^+–K^+ pump was modified, we clearly demonstrated that 5-HT reduces the AHP amplitude through the inhibition of the Na^+–K^+-ATPase (Catarsi & Brunelli, 1991). Since the effect of 5-HT develops with latency and lasts for 20–30 min after washing out, we have investigated the possibility that intracellular second messengers might be involved in the effect of the monoamine.

In this paper we present data suggesting that cyclic AMP mediates the effect of 5-HT in reducing the AHP amplitude in T neurones through the inhibition of the Na^+–K^+ electrogenic pump.

METHODS

Animals and preparation

Adult leeches of the species *Hirudo medicinalis* were bought from a local supplier and kept at 15 °C in a well-aerated aquarium. The animals were anaesthetized with 10% ethanol in tap water and pinned, ventral side up, to the paraffin wax floor of a plastic chamber. The ventral nerve cord was exposed by cutting the body wall along the midline and opening the ventral sinus. A short chain of ganglia were isolated at midbody level and pinned to the bottom of a small recording chamber coated with Sylgard (Dow Corning, Midland, MI, USA).

Solutions

Unless otherwise stated, the following saline solution was used, both for surgery and electrophysiological recording, containing (mM): 115 NaCl, 4 KCl, 1·8 $CaCl_2$, 10 glucose, buffered to pH 7·4 with Tris-maleate. In one group of experiments, we added 0·1 mM $CdCl_2$ to the saline solution in order to block $g_{K,Ca}$. Drugs (from Sigma, St Louis, MO, USA) were freshly dissolved in the saline solution just before their application at the rate of 1·5 ml min^{-1}, using a peristaltic pump (ISCO, Gio de Vita, Rome, Italy). The solution containing the adenylate cyclase inhibitor RMI 12330A (Merrel, Cincinnati, OH, USA) was warmed up to 40 °C to facilitate dissolving.

In one series of analyses, the Na^+–K^+ electrogenic pump was inhibited by perfusing the ganglia with a modified solution in which 4 mM KCl was substituted by 4 mM NaCl. To reactivate the pump we used a solution in which 4 mM KCl was replaced by 4 mM CsCl.

Electrophysiological recording

The AHP in sensory neurones was induced by trains of intracellular depolarizing pulses (200 ms, 2–3 Hz, 25 s duration). The discharge frequency of each series of trials was kept constant by adjusting the amount of current injected (0·8–1·2 nA). Microelectrodes filled with 4 M potassium acetate with resistances ranging from 50 to 80 MΩ were used for intracellular recordings and stimulations. The resting potential was constantly monitored and the input resistance was evaluated by injecting 0·5 nA hyperpolarizing pulses. To increase the activity of the Na^+–K^+ pump, in one group of experiments, we impaled the neurones with microelectrodes containing 3 M sodium acetate.

The involvement of second messengers on the modulation of AHP was examined using double-barrelled microelectrodes, with resistance ranging between 60 and 80 MΩ; one section was filled with 4 M potassium acetate and the other with 1 M cyclic AMP, cyclic GMP or AMP. Hyperpolarizing pulses (250 ms, 10 nA intensity, 0·5 Hz) were applied intracellularly for 60 s to inject the second messengers into the T cell soma.

Only cells with a resting potential of at least −40 to −50 mV, an input resistance greater than 30 MΩ and action potentials of 60–80 mV were selected. The AHP is very sensitive to the quality of impalement. Therefore, only cells with an AHP of at least 10–15 mV were used for the analyses.

All the experiments were displayed on the screen of a storage oscilloscope and collected on a video recorder connected to a pulse code modulator (PCM 501 ES, Sony).

Statistical analyses

The Mann–Whitney non-parametric test was used. This test is applicable when the normal Gaussian distribution may not be satisfied, as in handling data expressed as percentages (Belardetti, Biondi, Colombaioni, Brunelli & Trevisani, 1982; Belardetti, Biondi, Brunelli, Fabri & Trevisani, 1983).

RESULTS

Effect of 5-HT on sensory neurones

We first tested the effect of 5-HT on the AHP of the three types of sensory neurones located in each segmental ganglion of the leech. Application of 50 μM 5-HT for 10 min, caused a clear cut reversible reduction (up to 77%) of the AHP amplitude in T (tactile) neurones ($P < 0{\cdot}001$), consistent with previous data (Belardetti *et al.* 1984; Catarsi & Brunelli, 1991). However only a small reduction (about 10%) was detected in P (pressure sensitive) cells and no reduction at all was observed in N (nociceptive) neurones (Fig. 1).

The selective effect of 5-HT on the AHP of T neurones is in agreement with previous observations that 5-HT inhibits the Na^+–K^+ electrogenic pump (Catarsi & Brunelli, 1991). Since the depression of AHP amplitude in T cells is sustained (more than 30 min after wash) and develops with long latency (the peak of the effect is 20 min after the beginning of 5-HT application), we performed several experiments to study the possible involvement of intracellular second messengers.

Forskolin application on AHP amplitude

Application of 50 μM forskolin (an adenylate cyclase activator) for 10 min reduced the AHP amplitude by about 25% ($0{\cdot}02 < P < 0{\cdot}05$). The effect reached a peak at 10 min after perfusion with the drug (Fig. 2).

By combining 100 μM forskolin together with 10 μM theophylline (a phosphodiesterase inhibitor) the AHP amplitude was decreased by about 62% ($P < 0{\cdot}001$); the maximal effect was at 20 min after treatment with the two substances.

These experiments suggested that the depression of AHP amplitude brought about by 5-HT might be mediated by messengers of nucleotide type.

Effect of 3-isobutyl-1-methylxanthine (IBMX) treatment on AHP amplitude

In a group of experiments similar to the one described above, we applied 500 μM, 3 isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor (Beavo, Rogers, Crofford, Hardman, Sutherland & Newman, 1970; Colombaioni & Brunelli, 1988) for 10 min. A clear cut reduction of AHP amplitude (55%, $P < 0{\cdot}009$) was detected and a partial recovery from the effect was found after 15 min washing (Fig. 3).

Effect of 8-bromoadenosine-3′-5′ cyclic monophosphate (8-Br cyclic AMP) on AHP amplitude

After injecting a train of intracellular depolarizing pulses to induce an AHP, we perfused the ganglia with 100 μM 8-Br cyclic AMP, a membrane-permeant and phosphodiesterase-resistant analogue of cyclic AMP (Walsh & Byrne, 1989; Garcia-

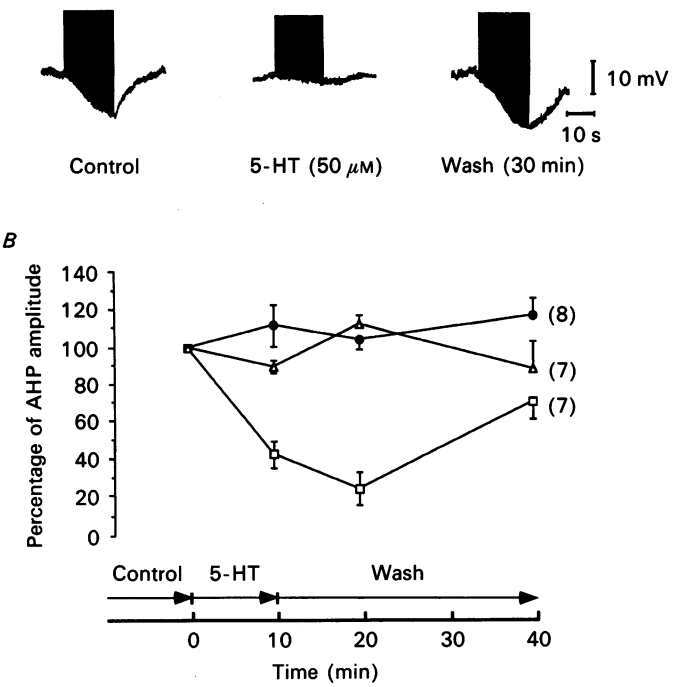

Fig. 1. Effect of 5-HT on sensory neurones. *A*, after a train of intracellular depolarizing pulses applied on a T neurone (control), 50 μM 5-HT was applied for 10 min: a clear cut, reversible reduction of the AHP amplitude was detected. The effect was long lasting. After a 30 min wash, AHP amplitude recovered almost completely. The recordings of action potentials are clipped at the peaks, in this and other figures. *B*, graph showing the mean ± S.E.M. of the AHP amplitude in experiments, conducted as described in *A* on all types of sensory neurones (T (□), P (△) and N (●)). Control of AHP amplitudes were taken as 100% in this and in the following figures. Number of cells tested is given in parentheses.

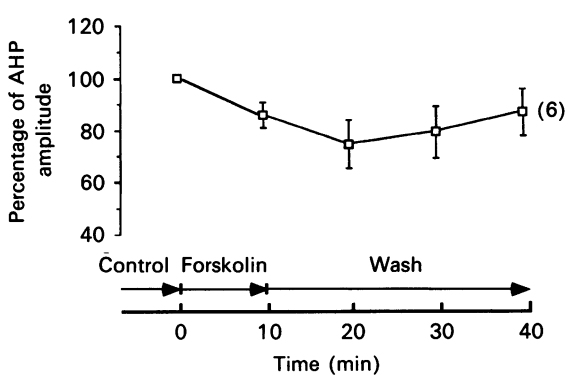

Fig. 2. Forskolin application on AHP amplitude. Graph showing the mean ± S.E.M. of the AHP amplitude in six cells treated with 10 min application of 50 μM forskolin.

Gil, Tongiorgi, Cattani, Cipollini & Brunelli, 1989*a*) for 10 min. A reversible 45% reduction of the AHP amplitude ($P < 0.006$) was found (Fig. 4) without significant modification of input resistance or resting potential.

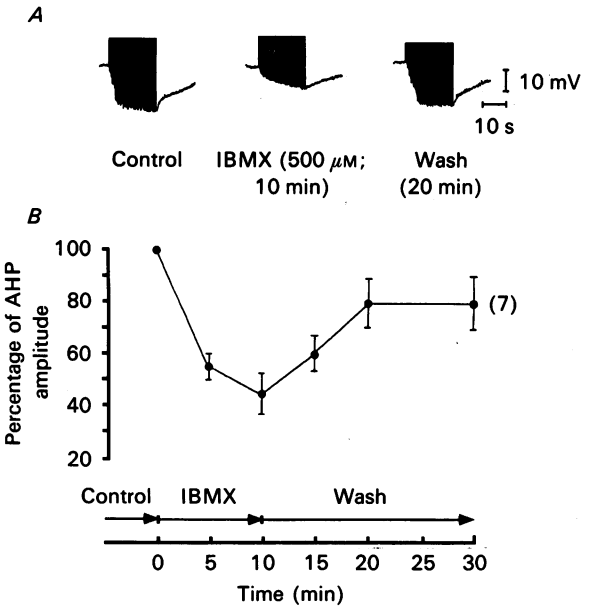

Fig. 3. Effect of IBMX on AHP amplitude. *A*, 10 min application of 500 μM IBMX brought about a reversible reduction of the AHP amplitude. *B*, graph showing the mean ± S.E.M. of the AHP amplitude in seven cells treated as shown in *A*.

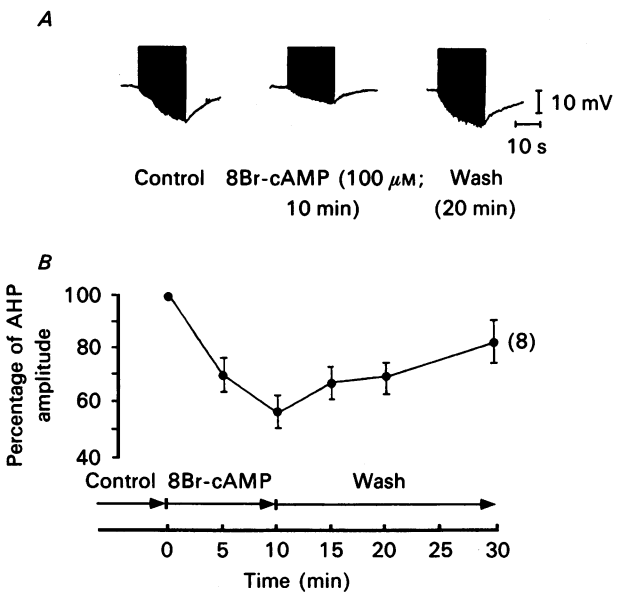

Fig. 4. Effect of 8-Br cyclic AMP on AHP amplitude. *A*, a train of depolarizing pulses was injected intracellularly into a T cell. The preparation was then perfused with 100 μM 8-Br cyclic AMP for 10 min and another depolarizing train was then delivered. A reduction of the AHP amplitude was observed. After 20 min washing the effect was reversed. *B*, graph showing the mean ± S.E.M. of the AHP amplitudes during 100 μM 8-Br cyclic AMP application in eight cells.

When 100 μM 8-Br cyclic AMP was applied together with 500 μM IBMX, the AHP reduction was also 55% ($P < 0.009$) but lasted longer.

Ionophoretic injection of second messengers into T cell

To obtain more direct evidence for the involvement of cyclic AMP, this nucleotide was injected by means of ionophoresis into T neurones (see Methods). This

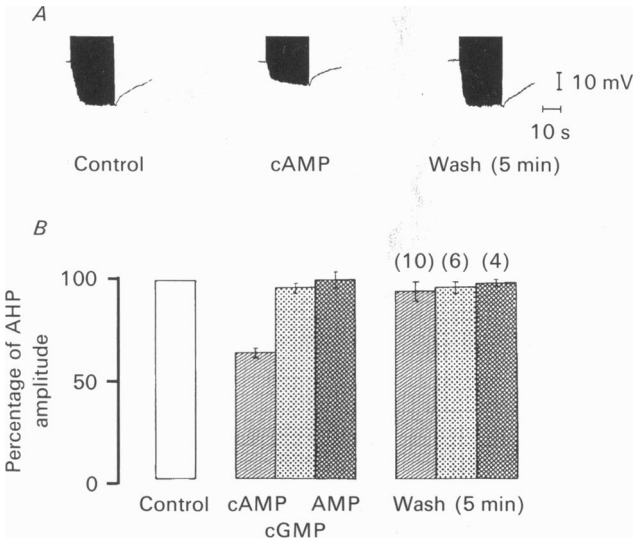

Fig. 5. Effect of ionophoresing second messengers on AHP amplitude. *A*, cyclic AMP was ionophoretically injected into a T neurone (see Methods), leading to a reversible reduction of the AHP amplitude. *B*, histograms showing the effects of cyclic AMP, cyclic GMP and AMP ionophoresis on the AHP amplitude of T cells in comparison with control values taken as 100%.

application reduced the AHP amplitude by 27% ($P < 0.001$), while cyclic GMP or AMP ionophoretic injections did not significantly alter either the AHP amplitude or other electrophysiological parameters (Fig. 5).

Effect of cyclic AMP analogue application on AHP amplitude in the presence of $CdCl_2$

Application of 0·1 mM $CdCl_2$ which inhibits $g_{K,Ca}$ from the extracellular side of the Ca^{2+} channel (Meech, 1978; Hagiwara, 1981; Madison & Nicholl, 1982; Stewart, Nicholls & Adams, 1989) reduced the AHP amplitude by up to 40% and increased the duration of the spike, together with the disappearance of the undershoot (Catarsi & Brunelli, 1991).

Taking the AHP amplitude as 100% after $CdCl_2$ treatment, a following incubation of 10 min with 100 μM 8-Br cyclic AMP together with 500 μM IBMX was still able to reduce reversibly the residual AHP amplitude by 40% ($P < 0.043$; Fig. 6). These data suggest that cyclic AMP may depress the AHP amplitude by inhibiting the

Na^+–K^+ electrogenic pump in a manner analogous to that observed following 5-HT application (Catarsi & Brunelli, 1991).

Effect of 5-HT on AHP amplitude after application of an adenylate cyclase inhibitor (RMI)

In order to verify that the reduction of the AHP caused by 5-HT was really mediated by the second messenger cyclic AMP, an experiment was performed in

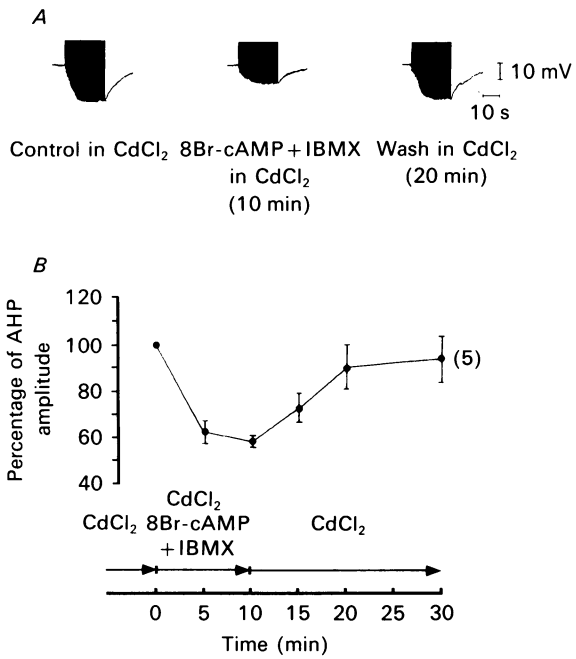

Fig. 6. Effect of 8-Br cyclic AMP + IBMX on AHP amplitude in the presence of $CdCl_2$. *A*, 0·1 mM $CdCl_2$ was applied for 10 min before inducing the AHP by intracellular depolarizing pulses. A 10 min application of 100 μM 8-Br cyclic AMP and 500 μM IBMX in $CdCl_2$ resulted in a further reversible reduction of the AHP amplitude. *B*, graph showing the mean ± S.E.M. of the AHP amplitude in five cells treated following the experiment shown in *A*.

which the effect of 5-HT was tested 15 min after perfusion with 100 μM RMI 12330A, an adenylate cyclase inhibitor in the leech (Biondi, Belardetti, Brunelli, Portolan & Trevisani, 1982; Pareschi, Portolan, Ferretti & Biondi, 1987; Biondi, Campi, Pareschi, Portolan & Ferretti, 1990). A 10 min perfusion with 50 μM 5-HT reduced the AHP amplitude by about 77% (Belardetti *et al.* 1984; Catarsi & Brunelli, 1991) and this depression was still present after 30 min washing. In the presence of RMI 12330A treatment, the 5-HT effect was reduced to 21% ($P < 0{\cdot}001$; Fig. 7) and disappeared completely after a 30 min wash.

In the presence of 0·1 mM $CdCl_2$, to remove the $g_{K,Ca}$ component, RMI 12330A application almost completely inhibited the effect of 5-HT ($P < 0{\cdot}001$; Fig. 7*A*). This result clearly confirmed that 5-HT is acting on the Na^+–K^+ electrogenic pump.

Effect of 8-Br cyclic AMP and IBMX on the response generated by intracellular injection of Na^+ ions in T cells

In order to test the possibility that cyclic AMP might depress the AHP through an inhibition of the Na^+–K^+ electrogenic pump, we carried out experiments in which the activity of the Na^+–K^+-ATPase was altered.

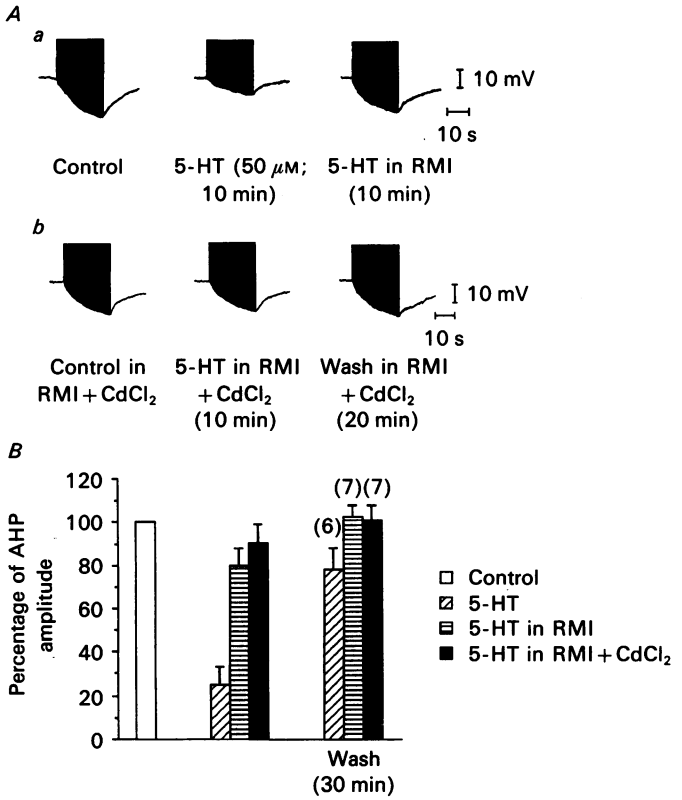

Fig. 7. Effect of 5-HT on AHP amplitude after application of the adenylate cyclase inhibitor RMI. *A a*, control experiments: 10 min application of 5-HT produced a decrease of AHP amplitude; this effect is blocked in the presence of 100 μM RMI 12330A. *A b*, after a 15 min application of 100 μM RMI 12330A in 0·1 mM $CdCl_2$, a train of intracellular depolarizing pulses was injected in order to elicit AHP. This value has been taken as the control. A 10 min perfusion with 50 μM 5-HT only slightly reduced the AHP amplitude. *B*, bar graphs showing the effect of 50 μM 5-HT, 50 μM 5-HT in 100 μM RMI 12330A and 50 μM 5-HT in 100 μM RMI + 0·1 mM $CdCl_2$. In the absence of the adenylate cyclase inhibitor, the reduction of the AHP amplitude was about 77% and a residual effect was still evident after 30 min of washing; with RMI 12330A treatment only an early reduction of the AHP (about 21%) could be detected, which disappeared almost completely in $CdCl_2$.

When T neurones were impaled with a 3 M sodium acetate microelectrode, a clear hyperpolarization could be observed due to the activation of the Na^+–K^+ electrogenic pump following the leakage of Na^+ into the cell (Jansen & Nicholls, 1973; Catarsi & Brunelli, 1991). The application of 100 μM 8-Br cyclic AMP and 500 μM IBMX

reversed the hyperpolarization into a depolarization (Fig. 8*A*). When the ganglia were perfused with 100 μM 8-Br cyclic AMP and 500 μM IBMX for 10 min before penetrating T cells using sodium acetate microelectrodes, we obtained a depolarization of about 8 mV after 5 min, while in the control Na^+ injection

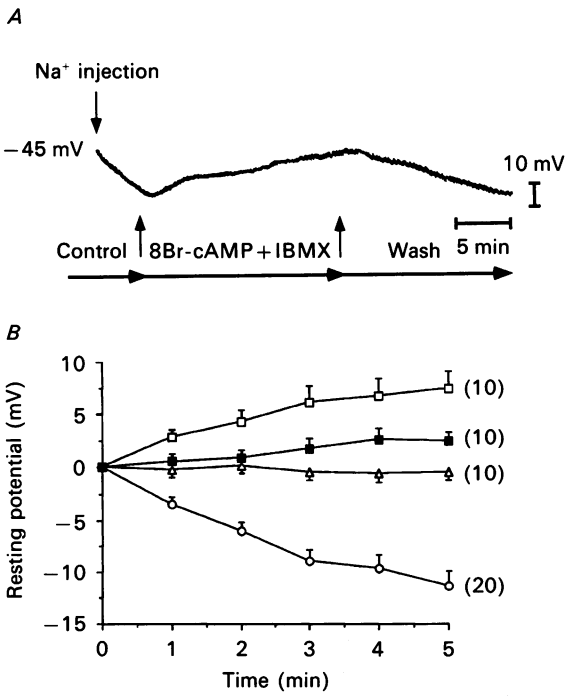

Fig. 8. Effect of 8-Br cyclic AMP+IBMX on the intracellular injection of Na^+ in T neurones. *A*, membrane potential of a T neurone after penetration with a 3 M sodium acetate microelectrode. A hyperpolarization due to the activation of the electrogenic pump can be seen. Perfusion with 100 μM 8-Br cyclic AMP+500 μM IBMX converted the hyperpolarization into a reversible depolarization. *B*, means ± S.E.M. of the variation in resting potential; ○, during 3 M sodium acetate injection; △, during 3 M potassium acetate injection; ■, during 3 M sodium acetate injection after 10 min of perfusion with 500 μM IBMX; □, during 3 M sodium acetate injection after 10 min of application of 100 μM 8-Br cyclic AMP and 500 μM IBMX. All the changes are referred to the initial level of resting potential.

experiments we observed a hyperpolarization of about 12 mV ($P < 0{\cdot}001$; Fig. 8*B*). A smaller depolarization effect (about 2·5 mV) when using 500 μM IBMX alone was also observed (Fig. 8*B*). If potassium acetate microelectrodes were used, no variation in membrane voltage was noted (Fig. 8*B*). This effect can be explained by the inhibition by cyclic AMP of the Na^+–K^+-ATPase so that Na^+ can no longer be pumped out and thus a depolarization occurs.

Effect of perfusion with 8-Br cyclic AMP and IBMX on membrane voltage following application of CsCl

After perfusing the ganglia with a K^+-free solution (see Methods), a biphasic change of membrane voltage was observed: an early hyperpolarization due to a

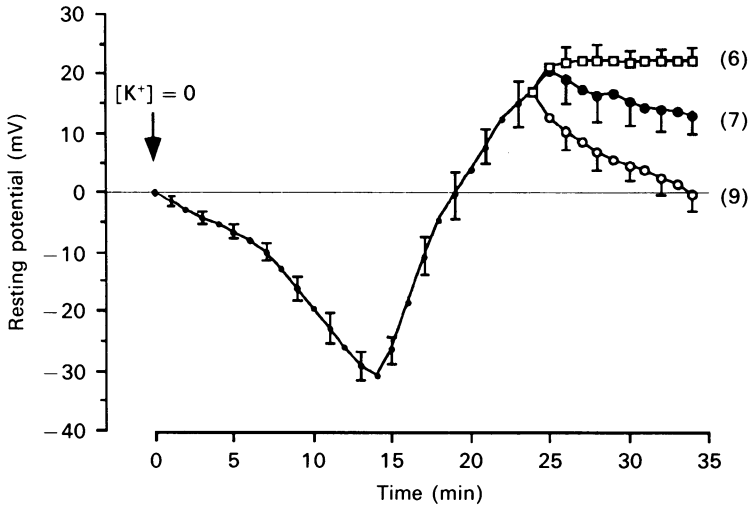

Fig. 9. Effect of 8-Br cyclic AMP + IBMX after application of CsCl. The small filled circles represent the means ± S.E.M. of the variation of the resting potential of T neurones (n = 22) perfused with K^+-free saline for about 25 min. Two phases are evident: a hyperpolarization followed by a depolarization. Symbols represent: □, sustained application of K^+-free solution; ○, after depolarization nine cells were perfused with saline in which KCl was completely replaced with CsCl. A repolarization can be observed. ●, after depolarization seven cells were treated for 10 min with 100 μM 8-Br cyclic AMP and 500 μM IBMX and then perfused with CsCl. The repolarization was significantly reduced.

larger K^+ outflow caused by the increased chemical gradient and a delayed depolarization, ouabain dependent and therefore mainly due to the inactivation of the electrogenic pump, which is sensitive to the external K^+ concentration (Schlue & Deitmer, 1984; Catarsi & Brunelli, 1991) (Fig. 9). At the end of the depolarization phase, K^+-free saline containing 4 mM CsCl, a Na^+–K^+-ATPase activator (Skou, 1960, 1965), was applied and the membrane potential repolarized to the initial value (Fig. 9; ○). Perfusion with 100 μM 8-Br cyclic AMP and 500 μM IBMX for 10 min before CsCl application, greatly reduced and slowed this repolarization ($P < 0{\cdot}001$; Fig. 9; ●).

DISCUSSION

A previous study has shown that 5-HT application or the intracellular electrical stimulation of the serotonergic Retzius cells depresses the AHP amplitude in T neurones of the leech which is mainly due to an electrogenic Na^+ pump (Baylor & Nicholls, 1969; Jansen & Nicholls, 1973). The 5-HT effect is dose dependent, is

reversible and is blocked by the antagonist methysergide and is not altered by 20 mM Mg^{2+} perfusion; besides, it does not affect the resting potential, the input resistance, the discharge threshold or the spike shape (Belardetti *et al.* 1984; Catarsi & Brunelli, 1991). In the present paper we have shown that the effect of 5-HT on the AHP amplitude is very small in P cells and absent in N neurones. It is well known that in these neurones the AHP is more dependent on $g_{K,Ca}$ (Baylor & Nicholls, 1969; Jansen & Nicholls, 1973; Van Essen, 1973). Therefore, these data are consistent with a serotonergic inhibition of the electrogenic pump as a mechanism to depress the AHP in T neurones.

The long latency of 5-HT action on the AHP amplitude in T cells and its sustained effect suggested that a chain of biochemical steps involving second messengers might be the mechanism leading to the AHP depression.

To verify this hypothesis we carried out three groups of investigations: (1) the study of AHP changes after a cyclic AMP analogue application; (2) the analysis of AHP depression by application of the adenylate cyclase activator and the phosphodiesterase inhibitor IBMX; (3) the effects of AHP modifications induced by intracellular injection of second messengers. Perfusion with 8-Br cyclic AMP, a membrane-permeable and phosphodiesterase-resistant analogue of cyclic AMP (Garcia-Gil *et al.* 1989*a*; Walsh & Byrne, 1989), mimicked the effect of 5-HT in reducing the AHP amplitude of T cells, without altering any electrophysiological membrane properties.

From these preliminary observations, it was difficult to ascribe the mechanism of action of 8-Br cyclic AMP to an inhibition of $g_{K,Ca}$; in fact no increase in input resistance was observed. A short circuit of the currents generating the AHP also failed to explain the action of the cyclic AMP analogue: a 45% reduction of the AHP amplitude should in fact result from an identical reduction of the input resistance. Moreover, a shunting action would affect the AHPs to the same degree, regardless of their amplitudes, while we have noted that larger AHPs are more depressed than the smaller ones, as happened with 5-HT (Belardetti *et al.* 1984). Possible 'remote' changes in conductance would have produced variations in the firing rate, but such changes were not observed.

Inhibition of basal phosphodiesterase activity with IBMX (Beavo, Rogers, Crofford, hardman, Sutherland & Newman, 1970; Colombaioni & Brunelli, 1988), have reduced the AHP amplitude in T neurones. The maximal reduction (55%) was larger than that provoked by 8-Br cyclic AMP. This may be partly explained by a 10% reduction of input resistance during IBMX perfusion. Application of 8-Br cyclic AMP together with IBMX resulted in a reduction of the AHP amplitude by 55%.

The AHP reduction elicited by ionophoretic injection of cyclic AMP into T neurones, supports a direct effect of this second messenger in this cell; the specificity of action of cyclic AMP is demonstrated by the fact that cyclic GMP or AMP ionophoretic injection does not affect the membrane properties of T neurones (data not shown).

In order to link the effect of AHP depression induced by cyclic AMP with the analogous action observed after 5-HT application, we carried out further experiments with RMI 12330A, an inhibitor of adenylate cyclase (Biondi *et al.* 1982, 1990; Pareschi *et al.* 1987). This substance, especially when associated with $CdCl_2$ to remove

the $g_{K,Ca}$ component, blocked reduction of AHP amplitude by 5-HT, demonstrating that cyclic AMP mediates the effect of 5-HT. The small residual reduction provoked by 5-HT in RMI 12330A (about 21%) can be due to either an RMI inhibitory effect on phosphodiesterase (Biondi *et al.* 1990) which may lead to an increase of endogenous cyclic AMP in spite of the adenylate cyclase block, or a 5-HT effect on a receptor not linked to cyclic AMP. The existence of multiple 5-HT receptors in the leech has recently been found in P neurones (Sanchez-Armass, Merz & Drapeau, 1991).

The larger 5-HT effect (77% reduction) in comparison to that of 8-Br cyclic AMP and/or IBMX (55%) could be explained by a secondary mechanism other than the main inhibition of the Na^+–K^+ electrogenic pump, that might be partly involved in the reduction of the AHP by 5-HT.

A direct inhibition of the Na^+–K^+-ATPase by cyclic AMP is evident by the fact that intracellular Na^+ injection after treatment with IBMX, 8-Br cyclic AMP or both caused a depolarization instead of the normal ouabain-sensitive hyperpolarization, because the Na^+ ions cannot be pumped out for the inhibition of the electrogenic pump. In addition during Na^+ injection the perfusion with the two drugs transformed the normal hyperpolarization in a reversible depolarization. Similar conclusion emerges from the experiments with Cs^+ after K^+-free perfusion.

Summing up, results show that cyclic AMP reduces the AHP amplitude in T sensory neurones and mediates the effect of 5-HT. The mechanism underlying this modulation involves inhibition of the Na^+–K^+ electrogenic pump. Such an effect provides evidence for a novel mechanism of synaptic transmission through which neurotransmitters, by means of second messengers, instead of acting onto passive membrane ionic channels, may modulate the electrogenic pump. This is supported by the inhibitory action of some neurotransmitters on electrogenic pumps described recently in other systems (Bertorello, Hopfield, Aperia & Greengard, 1990; Aperia, Fryckstedt, Svensson, Hemmings, Nairn & Greengard, 1991). In fact, examples of positive modulation of the electrogenic pump activity by neurotransmitters have previously been found (Thomas, 1972; Kuba & Koketsu, 1979; Phillis & Wu, 1981), but inhibitory effects have been investigated only from a biochemical point of view (Lingham & Sen, 1983; Mourek, 1988; Bertorello *et al.* 1990).

It is known that cyclic AMP supports phenomena of neuronal plasticity and simple forms of learning in invertebrates (Brunelli, Castellucci & Kandel, 1976; Belardetti *et al.* 1982; Kandel & Schwartz, 1982; Brunelli, Demontis & Traina, 1985; Brunelli, Colombaioni, Demontis & Traina, 1986). In *Hirudo medicinalis* cyclic AMP has been shown to play a role in mediating both 5-HT and dopamine action (Biondi *et al.* 1982; Pareschi *et al.* 1987) as well as protein phosphorylation (Garcia-Gil *et al.* 1989*a*; Garcia-Gil, Berton, Tongiorgi & Brunelli, 1989*b*).

The block of the Na^+–K^+-ATPase by 5-HT and cyclic AMP inhibits the AHP, reactivating the conduction of sensory stimuli; this phenomenon might be one of the mechanisms underlying dishabituation and sensitization of swim induction in the leech, both mimicked by 5-HT and cyclic AMP application (Brunelli *et al.* 1985, 1986; Catarsi, Garcia-Gil, Traina & Brunelli, 1990).

In contrast to *Aplysia*, in which sensitization is thought to be controlled by a serotonergic effect on all the sensory cells terminals, in the leech, 5-HT, through cyclic

AMP increase, might produce behavioural sensitization by relieving conduction block and increasing the traffic of action potentials along the sensory fibres. This, in turn, would increase the synaptic efficacy in all the areas of the follower cells. By acting on specific sites on the cell, the efficacy of synaptic output of many terminals can be controlled. This hypothesis agrees with the recent finding that conduction block may act as a switch in the central nervous system, altering the sensory neurone's pattern of synaptic transmission to different postsynaptic cells, depending on the area of the receptive field that is stimulated (Gu, 1991).

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