Br. J. Pharmac. (1985), **84**, 221–225

Muscarine and luteinizing hormone releasing hormone attenuate adrenaline induced hyperpolarization in amphibian sympathetic ganglia

Peter A. Smith & Jeffrey A. Zidichouski

Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

1 The adrenaline-induced hyperpolarization (Ad_H) and the responses evoked by muscarine and luteinizing hormone releasing hormone (LHRH) were recorded from neurones in amphibian sympathetic ganglia by means of the sucrose gap technique.

2 The amplitude of the Ad_H was reduced when 'M-channel' closure was promoted by superfusion of LHRH or muscarine.

3 4-Aminopyridine (4-AP, 1 mM) antagonized the Ad_H, but not the depolarization evoked by muscarinic agonists. This implies that the channels involved in the electrogenesis of the Ad_H have different pharmacological properties from 'M-channels' and that the Ad_H is not generated by the opening of 'M-channels' outside their normal voltage range.

4 Possible explanations for the attenuation of the Ad_H by muscarine and LHRH might be that (i) intracellular biochemical changes produced by these substances somehow interfere with the generation of the Ad_H or that (ii) muscarine and LHRH have allosteric interactions with the adrenoceptor mediating the Ad_H.

Introduction

Previous results obtained in this laboratory have suggested that the adrenaline-induced hyperpolarization (Ad_H) of amphibian sympathetic neurones might be generated by an increase in membrane permeability to K^+ (Smith, 1984). As reviewed by Brown *et al.* (1982), four different types of outward K^+ currents exist in amphibian sympathetic neurones. These are (i) I_K, a voltage sensitive 'delayed rectifier' K^+ current which is blocked by tetraethylammonium (TEA). (ii) I_c, a non-inactivating Ca^{2+}-sensitive K^+ current which is blocked by divalent cations such as Cd^{2+}, Mn^{2+} and Co^{2+} as well as externally applied TEA. (iii) I_A, a transient K^+ current which is kinetically similar to the 'A current' found in invertebrate neurones but is only weakly sensitive to 4-aminopyridine (4-AP) and (iv) I_M, a non-inactivating voltage-sensitive 'M-current' which is blocked by Ba^{2+}, muscarinic agonists and luteinizing hormone releasing hormone (LHRH). Closure of 'M-channels' by acetylcholine (ACh) is at least partly responsible for the generation of the slow excitatory post synaptic potential (e.p.s.p.) in ganglionic B cells (Kuffler & Sejnowski, 1983). In addition, a fast, rapidly decaying, Ca^{2+}-sensitive K^+ current, which may actually be a component of I_c, seems to be involved in the repolarization of the action potential (Adams *et al.*, 1982b; MacDermott & Weight, 1982). These different K^+ currents presumably reflect the existence of several types of pharmacologically distinct K^+ channels in amphibian sympathetic neurones. In an attempt to test further the hypothesis that the Ad_H may result from an increase in potassium conductance (G_K) (Smith, 1984) we tested the effects of various K^+ channel blockers on the Ad_H. This paper describes one particularly interesting aspect of the results which emerged from this series of experiments.

Methods

Paravertebral sympathetic ganglia were removed from small, pithed bullfrogs and arranged for sucrose gap recording as described previously (Smith, 1984). All drugs except LHRH were applied by superfusion. Responses to LHRH were evoked by slowly injecting 1 ml of Ringer solution containing the peptide from a syringe barrel which was attached to one of the three

way taps which made up the superfusion system. The composition of the desipramine (DMI) Ringer solution used for all experiments was NaCl 100 mM, KCl 2 mM, $CaCl_2$ 1.8 mM, Tris-HCl buffer (pH 7.2) 16 mM, DMI 0.5 μM and (+)-glucose 1 g l^{-1}. In experiments where methacholine (MCh) was used to evoke muscarinic responses, 70 μM (+)-tubocurarine chloride (TC) was included in the Ringer solution to limit activation of nicotinic receptors. All drugs were purchased from the Sigma Chemical Company, St Louis, MO, USA except for DMI (Pertofrane) which was a kind gift from Geigy Pharmaceuticals, Dorval, PQ, Canada.

Results

The Ad_H was not blocked by TEA (10 mM) or by the Ca^{2+} channel blocker, Mn^{2+} (10 mM). However, when the response was recorded in the presence of substances which promote membrane depolarization by closure of M-channels, its amplitude was reduced. Muscarine (50 μM) reduced the amplitude of the Ad_H to 41.1 ± 5.2% of control (mean ± s.e., $n = 7$) and LHRH (0.1–0.5 mM) reduced it to 51.0 ± 8.2% of control ($n = 6$). Typical experiments are illustrated in Figure 1 (a and b). The initial hyperpolarization produced by muscarine results from hyperpolarizing muscarinic receptors on ganglionic C cells (Weight & Smith, 1980) and the slower depolarization results, at least in part, from closure of M-channels in ganglionic B cells (Adams *et al.*, 1982a; Brown *et al.*, 1982). Note that the Ad_H responses recorded during the depolarization elicited by muscarine (Figure 1a,2) or LHRH (Figure 1b,2) were considerably smaller than their respective control responses (Figure 1a and b). The amplitude of the Ad_H responses returned toward control amplitude following re-

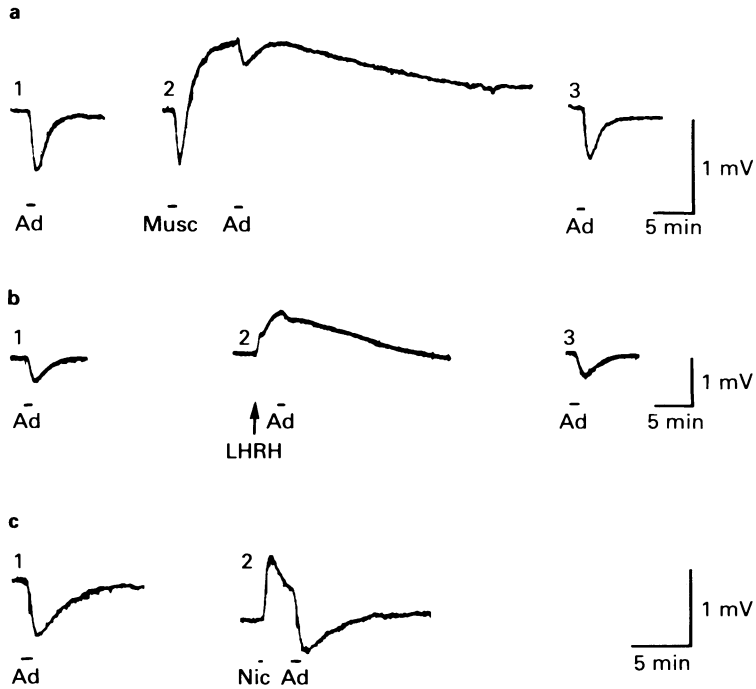

Figure 1 (a) Effect of muscarine on the adrenaline-induced hyperpolarization (Ad_H). (1) Control response to 1 μM adrenaline (Ad) (2). Response to 1 μM adrenaline evoked at the peak of the depolarization elicited by superfusion of 50 μM muscarine (Musc) for 30 s. Note reduction of amplitude of Ad_H under these conditions. (3) Response to 1 μM adrenaline recorded 125 min after the removal of muscarine. (b) Effect of luteinizing hormone releasing hormone (LHRH) on the Ad_H. (1) Control response to 1 μM adrenaline. (2) Response to 1 μM adrenaline evoked at the peak of the depolarization elicited by injecting 1 ml of 500 μM LHRH into the superfusion system. Note reduction of amplitude of Ad_H recorded under these conditions. (3) Response to 1 μM adrenaline recorded 27 min after the LHRH injection. (c) Effect of depolarization of membrane potential on the Ad_H. (1) Control response to 1 μM adrenaline. (2) Response to 1 μM adrenaline evoked during depolarization elicited by superfusion of 10 μM nicotine (Nic) for 15 s. Note that the Ad_H is not attenuated. Ringer solution used in all experiments contained 0.5 μM desipramine (DMI). Black bars under responses indicate duration of superfusion of agonists. Arrow indicates start of LHRH injection. Traces were from a rectilinear pen recorder.

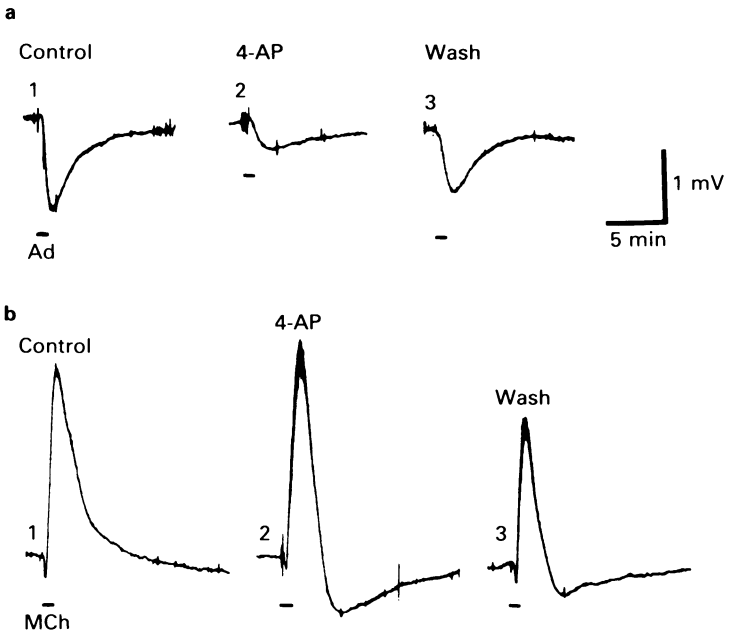

Figure 2 (a) Effect of 4-aminopyridine (4-AP) on the adrenaline induced hyperpolarization (Ad_H). (1) Control response to 1 μM adrenaline recorded in Ringer solution containing 0.5 μM desipramine (DMI) and (+)-tubocurarine (TC; 70 μM). (2) Response to 1 μM adrenaline recorded after 48 min superfusion of the ganglion with DMI/TC Ringer solution containing 1 mM 4-AP. (3) Response to 1 μM adrenaline recorded 45 min after resuming superfusion with DMI/TC Ringer solution. (b) Effect of 4-AP on the methacholine (MCh) response elicited in DMI/TC Ringer solution. (1) Control response to 1 mM MCh (30 s superfusion). (2) Response to MCh recorded after 60 min superfusion with Ringer solution containing 1 mM 4-AP. (3) Response to 1 mM MCh recorded 60 min after resuming superfusion with DMI/TC Ringer solution. The initial small hyperpolarization produced by MCh results from hyperpolarization of ganglionic C cells and the large depolarization results from depolarization of ganglionic B cells (cf. Weight & Smith, 1980). Traces in (a) and (b) from the same preparation. Black bars under responses indicate duration of superfusion with MCh or adrenaline. Traces were from a rectilinear pen recorder.

polarization of the ganglion (Figure 1a3 and b3). Since it has been shown that the amplitude of the Ad_H may be reduced by membrane depolarization (Koketsu & Nakamura, 1976), we also examined the effect of nicotine-induced depolarization on the Ad_H. At least with small depolarizations, which were comparable in amplitude to those produced by muscarine or LHRH, little or no attenuation of the Ad_H was seen (Figure 1c).

Since the Ad_H was not reduced by small membrane depolarizations yet was attenuated by substances which promote the closure of M-channels, it might be suggested that adrenaline promotes membrane hyperpolarization by opening M-channels outside their normal voltage range (cf. Adams *et al.*, 1982a). This interpretation seems unlikely in the light of the following result which suggests differences in the pharmacological properties of adrenaline-activated channels and M-channels. We found that 4-AP (1 mM, 30 min superfusion) reduced the amplitude of the Ad_H to $24.9 \pm 16.5\%$ of control ($n = 7$) yet did not antagonize muscarinic responses. A typical experiment is illustrated in Figure 2 (a and b). Although 4-AP drastically altered the shape of the muscarinic depolarization elicited by methacholine (MCh, 1 mM), no reduction in amplitude of the depolarizing phase of this response was seen at a time when the Ad_H was reduced to 28.3% of control.

Discussion

Although aminopyridines have been found to interact with several types of cation channels in excitable cells (Thomson, 1977; Thomson & Dryden, 1980; Rogawski & Barker, 1983) the simplest interpretation of the present results is that adrenaline produces membrane hyperpolarization by opening K^+ channels and these channels are 4-AP-sensitive. Since 4-AP blocks neither the depolarization due to

muscarinic receptor activation (Figure 2b) nor the M-current (Brown *et al.*, 1982) any 4-AP-sensitive K^+ channels which adrenaline might open are unlikely to be M-channels.

If this is the case, some explanation should be advanced for the apparent attenuation of the Ad_H by substances which promote M-channel closure. One possibility could be that this effect is simply an artifact of the sucrose gap recording technique used in the present experiments. As already mentioned, muscarinic agonists hyperpolarize ganglionic C cells and depolarize ganglionic B cells. Since the sucrose gap technique records a population response of all ganglionic neurones, it is possible that some C cells remain hyperpolarized during the depolarizing phase of the whole ganglion response. Since this hyperpolarization due to muscarinic receptor activation is associated with an increase in G_K (Horn & Dodd, 1981) it is possible that it could occlude any Ad_H responses occurring in C cells. This would result in attenuation of the total Ad_H recorded from the whole ganglion by means of the sucrose gap technique. We feel that the explanation is unlikely because the Ad_H is attenuated by both muscarinic agonists and LHRH. The latter substance promotes M-channel closure but does not hyperpolarize ganglionic C cells. Nevertheless, it would still be highly desirable to confirm that the amplitude of individual Ad_H responses in single neurones was attenuated during the action of muscarinic agonists or LHRH. Unfortunately, using the intracellular techniques available at present, we have only been able to record small and inconsistent responses to adrenaline. It is, therefore, not feasible to confirm the sucrose gap results using this technique.

Weight *et al.* (1978) have suggested that muscarinic receptor activation in sympathetic ganglia may produce two quite independent effects. These are (i) changes in membrane conductance, (ii) alterations in the intracellular levels of 'second messengers' such as cyclic nucleotides. Perhaps this latter type of effect could somehow alter cellular metabolism in such a way as to interfere with the electrogenesis of the Ad_H. Similar effects produced by LHRH could also account for the attenuation of the Ad_H by this substance. Alternatively, muscarine and LHRH could interact at an allosteric site on the adrenoceptor involved in the generation of the Ad_H. Indeed, Akasu *et al.* (1983) have suggested that LHRH may be able to act allosterically at ganglionic nicotinic receptors and reduce their sensitivity to ACh. Since there are now several examples of interactions between neurotransmitters, which may be independent of their immediate electrophysiological effects (Woodward *et al.*, 1979; Akasu *et al.*, 1983), it is possible that this type of phenomenon reflects a new general form of neuromodulatory mechanism.

Supported by the Alberta Heritage Foundation for Medical Research, the Alberta Mental Health Advisory Council and the Medical Research Council of Canada. We thank Dr Forrest F. Weight for useful discussions and Dr William F. Dryden for reading an early version of the manuscript.

References

ADAMS, P.R., BROWN, D.A. & CONSTANTI, A. (1982a). M-currents and other K^+ currents in bullfrog sympathetic neurones. *J. Physiol.*, **330**, 537–572.

ADAMS, P.R., CONSTANTI, A., BROWN, D.A. & CLARK, R.B. (1982b). Intracellular Ca^{2+} activates a fast voltage sensitive K^+ current in vertebrate sympathetic neurones. *Nature*, **296**, 746–749.

AKASU, T., KOJIMA, M. & KOKETSU, K. (1983). Luteinizing hormone-releasing hormone modulates nicotinic ACh-receptor sensitivity in amphibian cholinergic transmission. *Brain Res.*, **279**, 347–351.

BROWN, D.A., ADAMS, P.R. & CONSTANTI, A. (1982). Voltage sensitive K-currents in sympathetic neurones and their modulation by neurotransmitters. *J. autonom. Nervous System*, **6**, 23–25.

HORN, J.P. & DODD, J. (1981). Monosynaptic muscarinic activation of K^+ conductance underlies the slow inhibitory post synaptic potential in sympathetic ganglia. *Nature*, **292**, 625–627.

KOKETSU, K. & NAKAMURA, M. (1976). The electrogenesis of adrenaline hyperpolarization of sympathetic ganglion cells in bullfrogs. *Jap. J. Physiol.*, **26**, 63–77.

KUFFLER, S.W. & SEJNOWSKI, T.J. (1983). Peptidergic and muscarinic excitation at amphibian sympathetic synapses. *J. Physiol.*, **341**, 257–278.

MACDERMOTT, A.B. & WEIGHT, F.F. (1982). Action potential repolarization may involve a transient, Ca^{2+} sensitive outward current in a vertebrate neurone. *Nature*, **300**, 185–188.

ROGAWSKI, M.A. & BARKER, J.L. (1983). Effects of 4-aminopyridine on calcium action potentials and calcium current under voltage clamp in spinal neurons. *Brain Res.*, **280**, 180–185.

SMITH, P.A. (1984). Examination of the role of the electrogenic sodium pump in the adrenaline induced hyperpolarization of amphibian neurones. *J. Physiol.*, **347**, 377–395.

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

THOMSON, C.M. & DRYDEN, W.F. (1980). Development of membrane conductance of chick skeletal muscle in culture II. Effect of aminopyridines. *Can. J. Physiol. Pharmac.*, **58**, 606–611.

WEIGHT, F.F. & SMITH, P.A. (1980). Small intensely fluorescent cells and the generation of slow postsynaptic inhibition in sympathetic ganglia. In *Histochemistry and Cell Biology of Autonomic Neurons, SIF cells and Paraneurons. Advances in Biochemical Psychopharmacology*, Vol. 25, ed. Eranko, O., Soinila, S. & Pairvarinta, H. pp. 159–171, New York: Raven Press.

WEIGHT, F.F., SMITH, P.A. & SCHULMAN, J.A. (1978). Postsynaptic potential generation appears independent of synaptic elevation of cyclic nucleotides in sympathetic neurons. *Brain Res.*, **158,** 197–202.

WOODWARD, D.J., MOISES, H.C., WATERHOUSE, B.D., HOFFER, B.J. & FREEDMAN, R. (1979). Modulatory actions of norepinephrine in the central nervous system. *Fedn. Proc.*, **38,** 2109–2116.

(*Received July 18, 1984.*
Revised September 7, 1984.)