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Interactions between alinidine and responses to acetylcholine, dopamine and 5-hydroxytryptamine of specific *Helix* central neurones

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1 Intracellular recordings were made from identified neurones in the brain of the snail, *Helix aspersa*. The effect of alinidine on the excitatory and inhibitory responses to acetylcholine and dopamine and on the excitatory response to 5-hydroxytryptamine (5-HT) was investigated.

2 Alinidine was found to reduce the responses to acetylcholine and 5-HT and the excitatory response to dopamine but had no effect on the dopamine inhibitory response. pA_2 values were determined for alinidine antagonism to indicate relative potency. The pA_2 values against the excitatory responses of acetylcholine, dopamine and 5-HT were 5.8, 5.6 and 5.5 respectively. The pA_2 value against acetylcholine inhibition was 3.5.

3 From these studies it is suggested that alinidine interacts preferentially with the sodium ionophore, to a lesser degree with the chloride ionophore and not at all with the potassium ionophore on *Helix* central neurones.

Introduction

Alinidine (ST 567, N-allyl-clonidine) has been shown to exhibit bradycardiac effects on the sinus node of a number of mammals including rats, cats, rabbits and guinea-pigs (Kobinger, Lillie & Pichler, 1979; Millar & Vaughan Williams, 1981; Lillie & Kobinger, 1983). It would appear that the direct site of action of this compound is the sinus node. The anion permeability of this tissue has been reported to be particularly high (Seyama, 1979). From their investigations it was proposed by Millar & Vaughan Williams (1981) that alinidine might restrict current through anion-selective channels, implicating a possible interaction with chloride although they admit this conclusion is based largely on negative evidence since alinidine did not influence the fast inward current nor alter potassium conductance. However they observed that alinidine decreased the slope of the slow diastolic depolarization of sinus node cells and increased the duration but did not alter the amplitude of the action potentials. Millar & Vaughan Williams (1981) suggest that chloride may carry a substantial fraction of the current responsible for the slow diastolic depolarization.

The apparent lack of action on sodium or calcium inward currents is of interest in a compound which slows the heart, as is the suggestion that alinidine may be acting at a chloride ionophore. In order to try and resolve the site of action of alinidine we decided to examine its action on a preparation where it would be determined whether alinidine was acting specifically at an ionophore. The snail brain was chosen since it has identified neurones which respond to acetylcholine, dopamine and 5-hydroxytryptamine (5-HT) (Kerkut, Lambert, Gayton, Loker & Walker, 1975) and the ionic mechanism associated with each response has been investigated (Kerkut, Horn & Walker, 1969; Piggott, 1976; Chad, Kerkut & Walker, 1979; Bokisch & Walker, unpublished). The excitatory responses to acetylcholine, dopamine and 5-HT are associated with an increase in sodium conductance while the inhibitory responses to acetylcholine and dopamine are associated with increases in conductance to chloride and potassium respectively. It is therefore possible to look for specific interactions at either the ionophore or receptor level.

Methods

All experiments were performed on central neurones from the brain of the garden snail, *Helix aspersa*, which were collected locally and maintained in the laboratory. The preparation was dissected as described by Walker 1968) although in some cases a

small slit was made in the inner connective tissue to aid impalement with the microelectrode. The isolated ganglia were mounted on a glass slide, in an experimental bath of 5 ml volume. Identified neurones in the suboesophageal ganglia were impaled with glass microelectrodes, resistance 10–20 MΩ, filled with molar potassium acetate. Conventional electrophysiological recording techniques were employed but in the voltage clamp experiments, a Dagan 8100 single electrode voltage clamp system was used. Permanent records were made on a Hewlett Packard pen recorder.

The alinidine was added directly to the bath in a volume of 1 ml of Ringer solution and allowed to equilibrate for 3 to 5 min before testing. Concentrations given in the text are the final bath concentration values. The putative transmitters were ionophoresed directly onto the soma of the impaled neurone from a second micropipette positioned close to the cell soma. The compounds were ionophoresed at a concentration of 0.5 M at pH 4.5 except in the case of 5-HT bimaleate where 0.25 M was used. All the compounds were ejected as cations. The composition of the Ringer used was (mM) NaCl 80, KCl 4, $CaCl_2$ 7, $MgCl_2$ 5 and Tris-HCl 5, with a final pH of 7.4.

The pA_2 values were calculated from log dose-response curves constructed from the responses following ionophoretic application of the agonist. The pA_2 was calculated using the formula:

$$pA_2 = \log \frac{DR - 1}{[I]}$$

where [I] is the concentration of antagonist used and $DR = A_2/A_1.A_1$ is the dose of agonist applied to give a 50% maximal response and A_2 is the dose of agonist required to give the same size of response in the presence of the antagonist. The method is based on that devised by Schild (1947).

The alinidine (ST 567) was a gift from Boehringer Ingelheim. The other chemicals used in this study were: acetylcholine chloride (BDH); dopamine hydrochloride (SIGMA); 5-hydroxytryptamine bimaleate (Koch-Light).

Results

All experiments were performed on identified central *Helix neurones*, the following cells being used: E1, E2, E4, E21, F1, F2, F5 and F6 (Kerkut *et al.*, 1975). These cells were chosen since we wished to examine the effect of alinidine against a range of excitatory and inhibitory responses. The data are summarised in Table 1. Three excitatory, sodium-dependent, responses were used and in each case alinidine reduced the response. Figure 1b shows the effect of 0.87 μM alinidine on a dose-response curve to acetylcholine depolarization. This concentration of alinidine reversibly displaced the curve to the right. Alinidine also produced an increase in the duration of the response, prolonging both the time to peak and the decay phase of the response (Figure 2). The effect of alinidine was also examined on cells under voltage clamp and an example is shown in Figure 3. In this experiment, the membrane potential was held at −60mV. Figure 3a shows the currents obtained to increasing ionophoretic doses of acetylcholine. In the presence of 8.7 μM alinidine for 3 min, the inward sodium current was depressed (Figure 3b) and at this concentration of alinidine, the maximum current was depressed. Following washing for 10 min, the responses to acetylcholine returned to control values (Figure 3c). From the voltage clamp experiments, a pA_2 value of 5.4 was obtained for the acetylcholine excitatory response which compares favourably with the value of 5.8 obtained from membrane voltage measurements (Table 1). Similar pA_2 values were obtained in the cases of dopamine (pA_2 5.6) and 5-HT (pA_2 5.5) excitation (Table 1). Alinidine had no effect on the fast inward sodium current of the action potential or the voltage activated sodium currents observed in cell F1.

Table 1 Table to summarise the action of alinidine on the excitatory responses to acetylcholine, dopamine and 5-hydroxytryptamine and the inhibitory responses to acetylcholine and dopamine on neurones in the snail brain

		Action of Alinidine	pA_2	n
Acetylcholine:	excitation	Block	5.8 ± 0.2	6
	inhibition	Block	3.5 ± 0.1	5
Dopamine:	excitation	Block	5.6 ± 0.3	2
	inhibition	No effect	—	5
5-Hydroxytryptamine:				
	excitation	Block	5.5 ± 0.01	2

pA_2 values were calculated to indicate the relative potency of alinidine as an antagonist against each response.

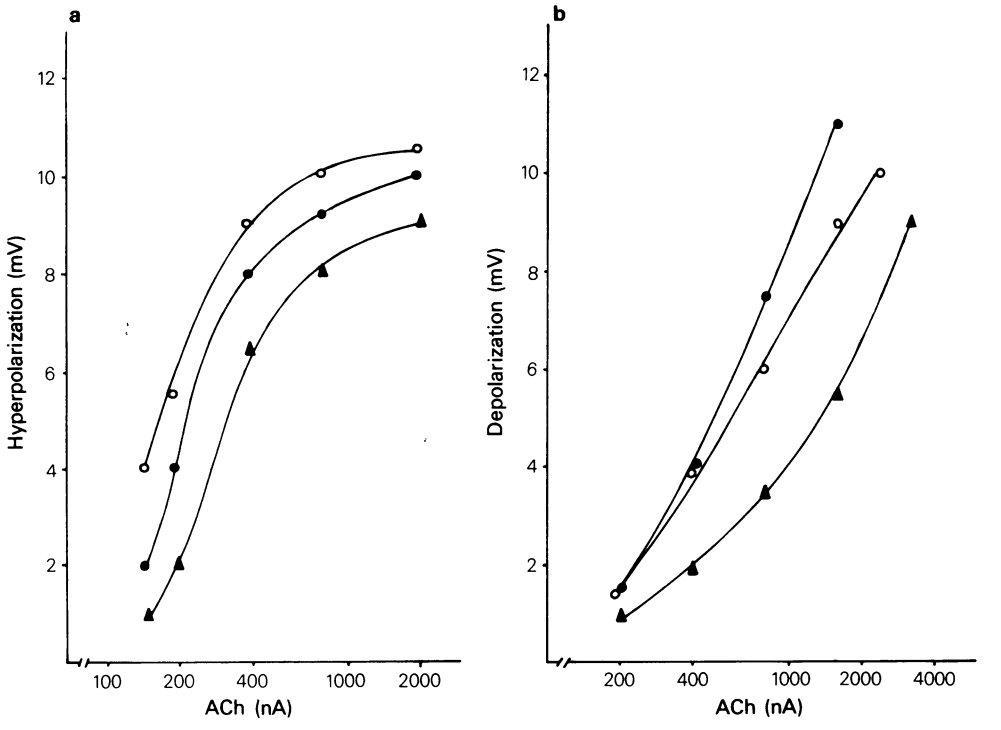

Figure 1 (a) Dose-response to acetylcholine on cell E4 which is inhibited by acetylcholine applied ionophoretically using 1 s pulses. Control responses shown in curve (●); in the presence of alinidine 87 μM, the curve is displaced to the right (▲); following washing for 5 min the response recovers and in addition shows slight potentiation (○). (b) Dose-response to acetylcholine on cell F6 which is excited by acetylcholine, applied ionophoretically using pulses of 1 s. Control responses are shown in curve (●); in the presence of alinidine 0.87 μM, the curve is displaced to the right (▲); following washing for 5 min the response recovers almost to control values but there was no indication of potentiation (○).

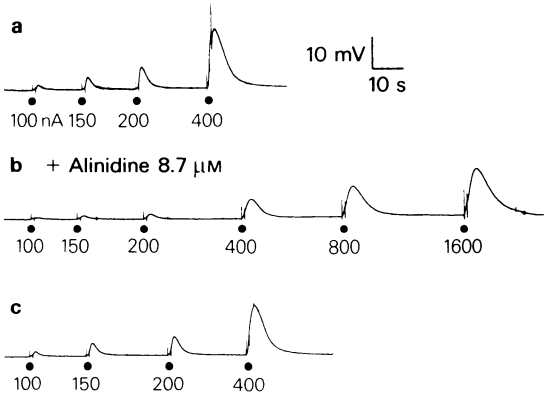

Figure 2 Intracellular recordings from cell E21 to show the effect of alinidine on an excitatory response to acetylcholine: (a) shows a control series of responses to increasing doses of acetylcholine applied ionophoretically; (b) shows the effect of alinidine 8.7 μM on the responses; (c) shows recovery of the acetylcholine responses following washing for 5 min. Each pulse of acetylcholine was applied for 1 s.

Concentrations of alinidine which reduced acetylcholine excitatory responses had no effect on acetylcholine inhibition. This inhibition is chloride-mediated. However, higher concentrations of alinidine, e.g. 87 μM, (Figure 1a) did displace the dose-response curve to the right but the maximum response was also reduced. This effect was reversible, as can be seen in Figure 1a, the recovery is often slightly potentiated compared with the control. A pA_2 value of 3.5 was obtained for the effect of alinidine against acetylcholine inhibition (Table 1). This value is considerably lower than the value of 5.8 obtained for alinidine against acetylcholine excitation. Finally the effect of alinidine was examined against dopamine inhibition which is a potassium-mediated event. Alinidine, up to a concentration of 440 μM, had no effect on dopamine inhibition though in cells which showed a biphasic response to dopamine, excitation followed by inhibition, the excitatory response to dopamine was depressed.

Under voltage clamp conditions the effect of alinidine was also examined on cell F2 where acetyl-

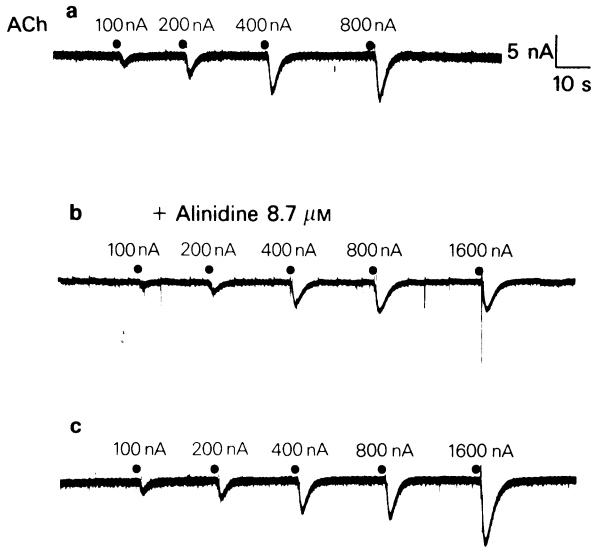

Figure 3 Intracellular recordings from cell E1 clamped at −60 mV to show the effect of alinidine on the inward current associated with the excitatory responses to acetylcholine applied ionophoretically; (a) control responses of 3.3, 6.1, 11.6 and 14.4 nA respectively; (b) shows the effect of alinidine 8.7 μM on the acetylcholine responses of 1.1, 2.8, 7.8, 9.4 and 9.4 nA respectively. The preparation was incubated with the alinidine for 3 min; (c) Shows the recovery after 10 min washing of the acetylcholine responses of 3.3, 5.6, 10, 12.2 and 20.5 nA respectively. Each pulse of acetylcholine was applied for 1 s.

choline increases the conductance to both sodium and chloride (Chad *et al.*, 1979). This cell has a variable reversal potential to acetylcholine, depending on the relative permeability of the two ions. In the presence of 8.7 μM alinidine, the reversal potential for this response was shifted to more negative values, indicating a possible interaction at the sodium ionophore.

Discussion

The present results indicate that, at least on the central neurones of one preparation, alinidine interacts with putative transmitter excitatory responses which are linked with sodium ionophores. Although alinidine does not affect the fast inward sodium current of *Helix* action potentials which supports the observation using mammalian sinus node tissue where alinidine apparently does not influence fast inward currents and hence these sodium ionophores (Millar & Vaughan Williams, 1981). The concentration of alinidine used in the present study and in the study of Millar & Vaughan Williams is comparable, though in terms of comparison with the likely plasma concentration of alinidine in man, it is likely that this is less than 1 μM (Kobinger, unpublished observation, quoted by Millar & Vaughan Williams, 1981). In the present study the only observation where the concentration of alinidine used was less than 1 μM, was where alinidine was antagonizing the excitatory actions of the putative transmitters.

Alinidine appears to show a preference for sodium ionophores associated with excitatory responses compared to either chloride or potassium ionophores of *Helix* neurones since the concentrations required for antagonist activity against chloride-mediated events is some 100 fold greater than for sodium events. While there appears to be no interaction between alinidine and potassium ionophores. The finding that alinidine is relatively inactive against *Helix* neuronal chloride ionophores contrasts with the suggestion from the experiments of Millar & Vaughan Williams (1981) using rabbit sinus node. It would be interesting to examine the effect of alinidine on other chloride ionophores to see if they provide any evidence for an interaction with alinidine.

The experiments described in the present study also show that alinidine does not appear to interact at the receptor level with putative transmitters, at least in the cases of acetylcholine and dopamine. This is supported by the finding that the antagonist potency shown by alinidine against the excitatory responses to acetylcholine, 5-HT and dopamine are similar, again supporting the notion that there is no selectivity at the receptor level. This agrees with the finding by Millar & Vaughan Williams (1981) that alinidine did not interact with the actions of either acetylcholine or isoprenaline on sinus tissue.

If as suggested from these results, alinidine acts preferentially on a certain population of sodium ionophores, i.e., those linked to excitatory drug responses, then alinidine should change the reversal potential of these drug responses (McCance, 1982). This type of analysis is easier with inhibitory than with excitatory responses. However there is one cell, F2, where both sodium and chloride are involved in the acetylcholine response (Chad *et al.*, 1979). Assuming that alinidine exhibits a preferential effect on sodium ionophores then in the presence of alinidine, the reversal potential for this response should move to a more negative value. In some preliminary experiments this does in fact occur which strengthens the proposal that alinidine acts at a level of the sodium ionophore.

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