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# ACTIONS OF NORADRENALINE RECORDED INTRACELLULARLY IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONES, *IN VITRO*

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## SUMMARY

1. CA1 pyramidal neurones were studied in rat *in vitro* hippocampal slices using standard intracellular and single-electrode voltage-clamp recording techniques to examine the actions of noradrenaline (NA).

2. NA had two different effects on the resting membrane potential of pyramidal neurones; either a hyperpolarization accompanied by a decrease in membrane input resistance, or less commonly, a depolarization accompanied by an increase in input resistance. In many cells, both effects, a hyperpolarization followed by a depolarization were observed. The depolarization was mediated by a noradrenergic $\beta$-receptor. The hyperpolarization was more difficult to characterize, but may result from $\alpha$-receptor activation.

3. NA reduced the amplitude and duration of the slow calcium-activated potassium after-hyperpolarization (a.h.p.) that follows depolarization-induced action potentials. This action of NA was mediated by $\beta_1$-noradrenergic receptors.

4. NA, in the presence of tetrodotoxin and tetraethylammonium, reduced the a.h.p. without reducing the size of the calcium action potential which preceded it. This was unlike the action of the calcium channel blocker, cadmium, which reduced the calcium action potential and the a.h.p. in parallel. Furthermore, NA did not reduce the amplitude of calcium or barium currents recorded under voltage clamp after blockade of potassium currents.

5. A functional consequence of this blockade of the calcium-activated a.h.p. was a reduction of the accommodation of action potential discharge such that the excitatory responses of the neurone to depolarizing stimuli, such as glutamate application or current passed through the recording electrode, were enhanced.

6. We conclude that the effects of NA on calcium-activated potassium conductance and on resting membrane potential can interact to increase the signal-to-noise ratio of hippocampal pyramidal neurone responsiveness.

## INTRODUCTION

The actions of noradrenaline (NA) in the central nervous system, and the hippocampus in particular, appear to be quite complex. Many previous studies have characterized the central action of NA as that of an inhibitory neurotransmitter. In

cerebellar Purkinje neurones (Hoffer, Siggins & Bloom, 1971; Siggins, Oliver, Hoffer & Bloom, 1971; Hoffer, Siggins, Oliver & Bloom, 1973; Freedman, Hoffer, Woodward & Puro, 1977), and hippocampal pyramidal cells (Biscoe & Straughan, 1966; Segal & Bloom, 1974*a*, *b*; Herrling, 1981; Langmoen, Segal & Andersen, 1981; Segal, 1981), NA has been reported to have inhibitory effects in that it slows the spontaneous discharge rate or causes a hyperpolarization in these cells. These effects have most often been ascribed to the action of NA at a $\beta$-adrenergic receptor (Hoffer *et al.* 1971; Segal & Bloom, 1974*a*; Segal, 1981) although $\beta$-receptor-mediated excitatory actions in these neurones have also been reported (Mueller, Hoffer & Dunwiddie, 1981; Mueller, Palmer, Hoffer & Dunwiddie, 1982; Basile & Dunwiddie, 1984).

Excitatory actions of NA, either direct or indirect, have also been described in several different neurones of the central nervous system. In the lateral geniculate nucleus (l.g.n.), NA application or locus coeruleus stimulation causes an $\alpha$-receptor-mediated excitation of neurones recorded extracellularly. This action may be caused either by increasing the responsiveness of l.g.n. neurones to excitatory transmitters, such as glutamate (Rogawski & Aghajanian, 1980), or by suppressing the activity of inhibitory interneurones (Nakai & Takaori, 1974; Kayama, Negi, Sugitani & Iwama, 1982). In the olfactory bulb, NA can cause an excitation of the mitral neurone by reducing inhibitory synaptic potentials (Jahr & Nicoll, 1982).

It has been proposed that NA may have actions on neurones unrelated to changes in resting membrane potential (Bloom, 1973; Moore & Bloom, 1979; Woodward, Moises, Waterhouse, Hoffer & Freedman, 1979). We have found a novel action of NA in the hippocampus which is similar in some respects to an action of NA on sympathetic ganglion neurones (Horn & McAfee, 1980). NA applied to the *in vitro* hippocampal slice causes a reduction in calcium-activated potassium after-hyperpolarizations in pyramidal neurones (Madison & Nicoll, 1982; Haas & Konerth, 1983). The reduction in this intrinsic inhibitory potential produces an increase in the excitability of pyramidal neurones (Madison & Nicoll, 1982). In this paper, we report the results of experiments which more fully characterize the actions of NA on hippocampal pyramidal neurones.

## METHODS

The methods used in this paper are similar to those used in other studies from this laboratory (Nicoll & Alger, 1981; Alger & Nicoll, 1982). Rat hippocampal slices, 400 $\mu$m thick, were cut and placed in a holding chamber for at least 1 h. A single slice was then transferred to the recording chamber and held between two nylon nets, submerged beneath a continuously superfusing medium which had been pre-gassed with 95 % $O_2$, 5 % $CO_2$. The composition of this medium was (mM): NaCl, 116·4; KCl, 5·4; $MgSO_4$, 1·3; $CaCl_2$, 2·5; $NaH_2PO_4$, 1·0; $NaHCO_3$, 26·2; glucose, 11. The temperature of the medium was maintained between 29 and 31 °C. Drugs were applied by addition to the superfusate, by ionophoresis (WPI 606 ionophoresis programmer), or by extracellular pressure application from a broken micropipette (Picosprizer, General Valve Co.). For the catecholamines, particular care was taken to minimize oxidation of the compounds (cf. Hughes & Smith, 1978). A stock in distilled water was made immediately prior to the experiment. For each application, the drug was added to oxygenated medium immediately before switching to the solution. For bath application of drugs, a latching solenoid valve was used (General Valve Co.) which allowed for rapid switching between superfusate solutions containing known concentrations of drugs, without disturbing the stability of intracellular recordings. Such application in the superfusing bath was the method of drug delivery in all experiments unless otherwise noted. Drugs used in this study,

which were obtained from the Sigma Chemical Co. unless otherwise indicated, were: (−)noradrenaline (arterenol) (hydrochloride and bitartrate), (−)isoprenaline HCl (isoproterenol), phenylephrine HCl, propranolol HCl, carbamylcholine chloride (carbachol), (−)adrenaline bitartrate, tetrodotoxin (TTX), tetraethylammonium chloride (TEA), cadmium chloride, glutamic acid monosodium salt, atenolol HCl, phentolamine mesylate (Regitine, Ciba-Geigy) and dobutamine (Dobutrex, Eli Lilly Co.). Butoxamine, clonidine and salbutamol were the generous gifts of Burroughs Wellcome Co., Boehringer Ingelheim Ltd. and Glaxo Group Ltd. respectively.

Conventional intracellular recording techniques were used for most of the experiments reported here. Intracellular electrodes were pulled from 'omega dot' borosilicate glass capillary tubing (1·2 mm o.d., 0·6 mm i.d.; Glass Co. of America), and were filled with either 2 M-potassium methylsulphate ($KMeSO_4$) (ICN Pharmaceuticals; electrode resistance 100–140 MΩ), or 3 M-potassium chloride (resistance 80–100 MΩ). In all experiments, $KMeSO_4$ was used in the electrode unless otherwise indicated. Neurones were stimulated by passing depolarizing current through the recording electrode using a standard bridge amplifier (WPI M-707).

During the course of some experiments, drug application caused small changes in the resting membrane potential of the cell. In these cases, the membrane potential was returned to control levels by passing steady direct current through the recording electrode before transient potentials, such as a.h.p.s, were recorded and before changes in membrane resistance were measured. This was done to eliminate any possible membrane potential-dependent changes in these measurements. Places where this was done are marked in the Figures with a bar labelled '−d.c.'. The amplitude of the slow component of the a.h.p. was measured relative to resting potential.

In many experiments, particularly those dealing with pharmacological characterization of the calcium-activated potassium a.h.p., cells were recorded in medium containing 1 μM-TTX and 5 mM-TEA. This was done routinely because in this condition the size of a.h.p., which followed a single current-evoked calcium action potential, tended to be large and extremely stable, allowing for easier and more definite assesment of drug-induced changes in this potential.

To measure the areas of calcium action potentials and a.h.p.s, a Nicolet 4094 digital oscilloscope was used. The area under the current-evoked calcium action potentials was taken from the point at which the membrane potential departed from base-line voltage to the point at which the repolarizing action potential-voltage crossed base line again. From this area was subtracted the area of the stimulus current-induced electrotonic potential remaining after complete blockade of the action potential by cadmium (100 μM). The area of the a.h.p. that extended below base-line voltage was taken from the point at which the repolarizing calcium action potential crossed the base-line voltage, to the point at which the decaying a.h.p. voltage again reached the base-line membrane potential.

To examine the effects of NA on calcium or potassium currents, single-electrode voltage-clamp techniques were used. Such recordings were made using a modified Dagan 8100 or an Axoclamp 2 (Axon Instruments) single-electrode voltage-clamp. Electrodes were pulled from thin-wall (1·2 mm o.d., 0·9 mm i.d.) 'omega dot' glass capillaries and were filled with either 3 M-KCl (resistance 25–80 MΩ), or with 3 M-caesium chloride (resistance 30–50 MΩ). Electrodes were coated to within approximately 50–100 μm of the tip with M-Coat D, air drying micro-electrode coating (Measurements Group Inc., Raleigh, NC), to reduce electrode capacitance. The voltage-clamp head stage output was continuously monitored and the switching frequency was adjusted to the maximum rate which still allowed the electrode voltage to settle completely between oscillations. A switching frequency of between 3 and 8 kHz was usually obtained (50% duty cycle when using the Dagan clamp, 30% with the Axoclamp) depending on the characteristics of the electrode employed. For measurement of currents through calcium channels, cells were recorded with caesium-chloride-filled micro-electrodes, the preparation was bathed in 0·5 μM-TTX, 30 mM-TEA, 15 mM-magnesium chloride and calcium was reduced to 1 mM. In some experiments the calcium in the solution was replaced with 1 mM-barium chloride. To make the barium containing solution, phosphate (as $NaH_2PO_4$) was removed from the medium and $MgCl_2$ replaced $MgSO_4$. This altered medium allowed the examination of inward currents which were relatively uncontaminated by outward currents. Inward calcium or barium currents were evoked by making depolarizing voltage steps from holding potentials between −30 and −40 mV. Net inward currents, elicited by depolarizing steps, were assessed by subtracting the leakage current resulting from a mirror hyperpolarizing step. These subtractions were executed on a Nicolet 4094 digital oscilloscope, and were made only in cases in which no voltage-dependent current could be observed during the

hyperpolarizing step. In other experiments measurements of the M-current, a voltage and muscarine-sensitive potassium current (Adams, Brown & Constanti, 1982; Halliwell & Adams, 1982), were made by recording the inward relaxation which occurred during hyperpolarizing steps from holding potentials around −30 to −40 mV, in cells bathed in standard medium and impaled with KCl-filled micro-electrodes. Only experiments where this inward relaxation was reduced by application of cholinergic agonists were used.

Results in this paper were recorded on a chart recorder (Gould 2400) and were photographed directly from the screen of an analogue oscilloscope (Tektronics 5113, Grass Kymograph Camera). Some data were recorded on a digital oscilloscope (Nicolet 4094) and plotted with a digital plotter (Hewlett-Packard 7470A). These results, and those in the following paper are based on recordings from over 500 pyramidal neurones with membrane potentials greater than −55 mV which were stable for at least 30 min.

## RESULTS

### *Effects of NA on the resting membrane potential of pyramidal neurones*

NA had two separate and distinct effects on the resting membrane potential of pyramidal neurones, either a hyperpolarization or a depolarization. In cells bathed in normal medium and recorded with $KMeSO_4$-filled micro-electrodes, NA application caused a hyperpolarization of the membrane potential 44% of the time ($n = 119$) which was usually accompanied by a decrease in resting input resistance (Fig. 1*C*). In 21% of these pyramidal neurones, NA caused a depolarization of the resting membrane potential which was accompanied by an increase in the membrane input resistance (Fig. 1*B*). In many of these cells (19%), both effects were observed, with a hyperpolarization followed by a depolarization (Fig. 1*A* and *B*). The remaining cells showed no changes in membrane potential when NA was applied. The responses in Fig. 1 are among the largest that we recorded and in general, these NA-induced changes in resting membrane potential were quite small, rarely exceeding 2–3 mV in amplitude. There was a great deal of variation between responses evoked by NA during repeated application in the same cell and also between applications in different cells. This made it very difficult to study these responses in a systematic manner, but nonetheless, some information on the characteristics of these NA-induced changes in resting membrane potential was obtained.

To determine the receptor subtype involved in the membrane potential effects, specific receptor agonists and antagonists were employed. In experiments using receptor antagonists, great care was taken to obtain stable and reproducible responses to NA before the antagonist was applied. The $\alpha$-receptor antagonist, phentolamine (10 $\mu$M) (Fig. 1*C*) reduced the amplitude of NA-induced hyperpolarizations in six of seven cells although the concentration needed to block the hyperpolarization was higher than that generally required to block $\alpha$-receptors. Cases in which phentolamine reduced a depolarizing response were not observed. The depolarizing response to NA was reduced by application of the $\beta$-receptor antagonist propranolol (0·3–10 $\mu$M) in five of seven cells (Fig. 1*B*, see also Fig. 3*B*). Cases in which propranolol reduced an NA-induced hyperpolarizing response were not observed. These data suggested that the NA-induced hyperpolarization was due to the activation of $\alpha$-receptors while activation of $\beta$-receptors caused a depolarization.

Receptor-selective agonists also caused changes in pyramidal cell resting potential. Hyperpolarizations were produced by phenylephrine (10 $\mu$M) in 70% of cells tested ($n = 27$) and by clonidine (10–100 $\mu$M) in 43% ($n = 16$). Even when these high

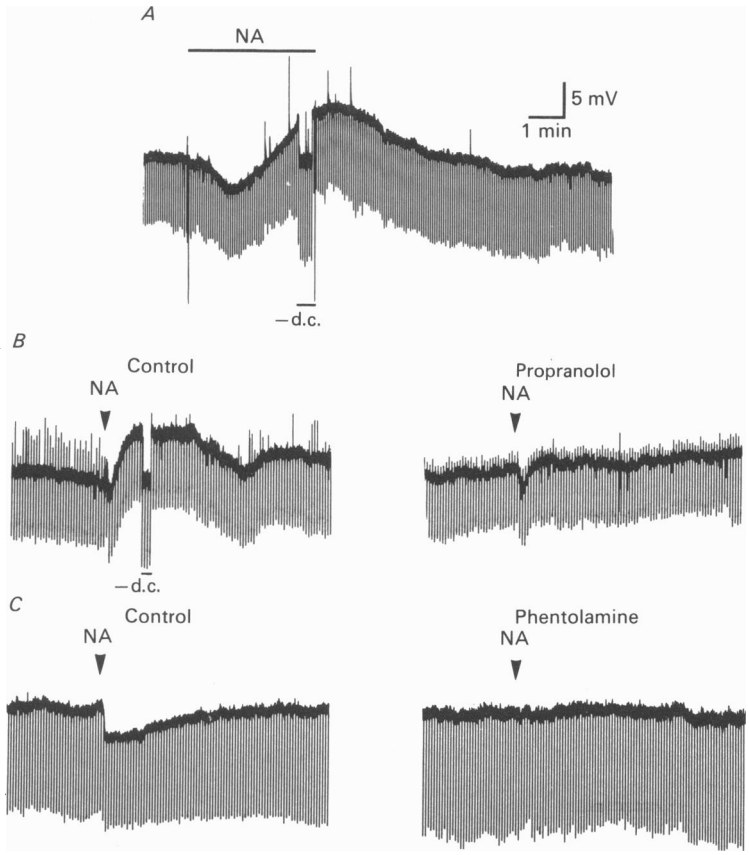


Fig. 1. Effects of noradrenaline (NA) on CA1 pyramidal cell resting membrane potential. Responses shown in *A* and *B* were recorded in the presence of TTX (1 μM), and TEA (5 mM). Responses shown in *C* were recorded in the presence of TTX (1 μM) alone. The responses in *B* and *C* were recorded with KCl-filled (3 M) micro-electrodes. Downward deflexions in these records are constant current hyperpolarizing pulses passed through the recording electrode to measure membrane resistance. Steady direct current was passed through the recording electrode at the places indicated (–d.c.) to repolarize the membrane to control levels. *A*, chart records of a biphasic action caused by bath application of NA (10 μM) onto a pyramidal cell. The period of application is indicated by the bar labelled 'NA' above the record. The membrane potential of this cell was −55 mV. *B*, chart records from another cell showing a biphasic change in resting membrane potential in response to an extracellular pressure application of NA. The depolarizing phase of this response was attenuated by application of 10 μM-propranolol. Membrane potential was −58 mV. *C*, chart records from another pyramidal cell showing a pure hyperpolarizing response to pressure application of NA. Application of phentolamine (10 μM) attenuated this hyperpolarizing response. The membrane potential of this cell was −56 mV.

concentrations (10–100 μM) of agonist were used, these effects were generally too small to allow for sub-classification of the receptor-type responsible for the hyperpolarization. Application of the β-agonist isoprenaline (1–10 μM) caused a depolarization in 82% of cells tested ($n = 40$). In those cells where membrane input resistance was measured, these depolarizations were accompanied by an increase in resistance. These

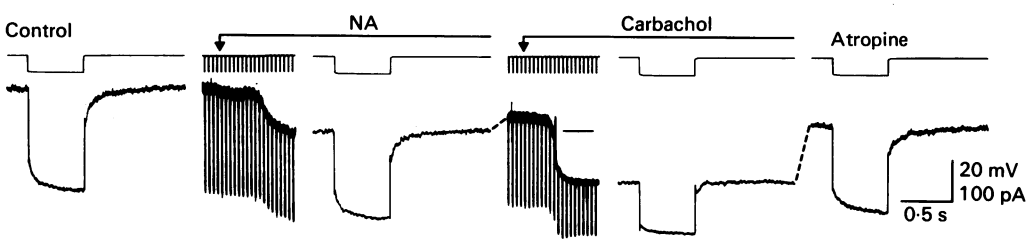


Fig. 2. Noradrenaline (NA) does not reduce the voltage-sensitive M-current ($I_M$). Shown are chart records of single-electrode voltage-clamp recordings taken from a CA1 pyramidal neurone. The membrane voltage record is shown above the current record. One second duration hyperpolarizing commands from a holding potential of −40 mV produced time-dependent inward relaxations in membrane current. NA (10 $\mu$M) produced an inward shift in holding current with an increase in membrane resistance but did not reduce this relaxation. Application of carbachol (10 $\mu$M) caused a further inward shift in holding current with an increase in resistance, and reduced the inward relaxation confirming that it was due to the voltage-dependent inactivation of $I_M$. Addition of 1 $\mu$M-atropine reversed the effects of carbachol.

results support the conclusion that the hyperpolarization of the membrane potential was caused by the activation of $\alpha$-receptors, and the depolarization was produced by activation of $\beta$-receptors.

Despite the small size and variable nature of the NA-induced changes in membrane potential, we have made a few observations which may provide evidence towards a mechanism for these effects. Both responses could be observed in cells recorded with KCl-filled micro-electrodes. Because such cells have a reversed chloride gradient which would reverse chloride-mediated responses (cf. Alger & Nicoll, 1980*b*), this suggested that both the hyperpolarization and the depolarization are mediated, at least in part, by changes in potassium conductance. When TEA (5 mM) was present in the medium, many fewer hyperpolarizations and more depolarizations were recorded during NA application ($n = 69$; hyperpolarizations 4%, depolarizations 71%, biphasic responses 16%, no effect 9%). This could suggest that the hyperpolarization was caused by the opening of a TEA-sensitive potassium channel. However, a chloride-dependent component of these responses could not be ruled out. Both the hyperpolarization and depolarization could be recorded in cells bathed in 1 $\mu$M-TTX which suggested that these are, at least in part, direct actions of NA on pyramidal cells. However, any possible chloride component, particularly of the hyperpolarization, could possibly involve an indirect mechanism since NA is known to increase the frequency of chloride-mediated spontaneous inhibitory potentials on pyramidal neurones (Madison & Nicoll, 1984*b*).

The depolarizing component of NA action appears to be mediated by a decrease in potassium conductance because it is associated with an increase in input resistance and is not reversed by chloride injection. One current which seemed a likely candidate to underlie this depolarization was the M-current, a non-inactivating, voltage-dependent, muscarine-sensitive potassium current which has been reported to be present in pyramidal cells (Halliwell & Adams, 1982). This possibility was tested by observing the effects of NA on the M-current recorded with a single-electrode voltage clamp. In Fig. 2, a pyramidal cell was voltage clamped at a holding potential

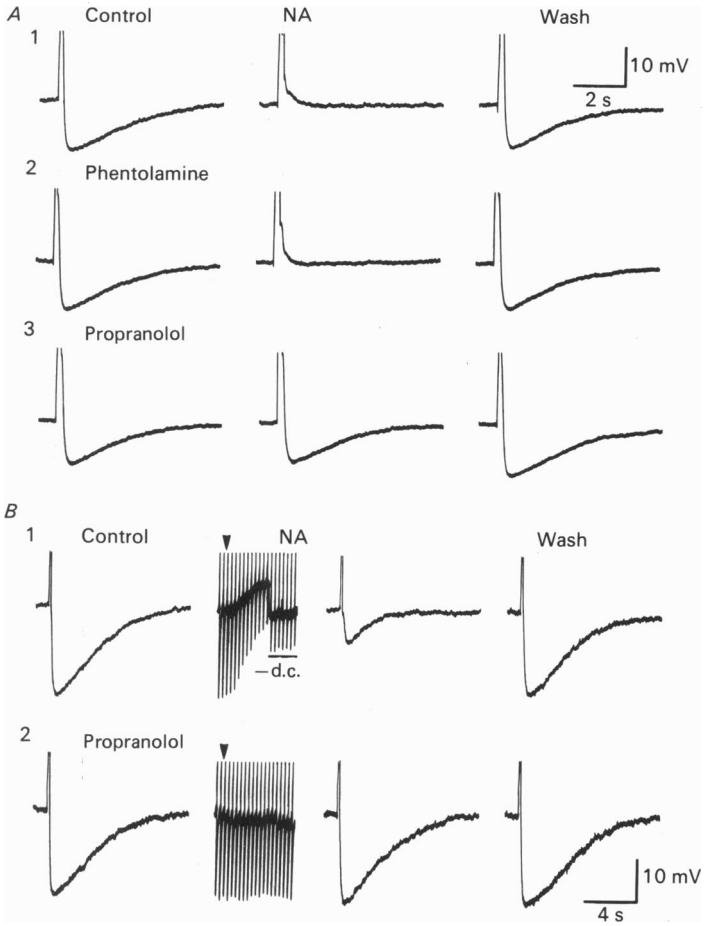


Fig. 3. Propranolol but not phentolamine prevents the blockade of the after-hyperpolarization (a.h.p.) by NA. CA1 pyramidal cells bathed in TTX (1 $\mu$M) and TEA (5 mM) were stimulated with a short duration current pulse to discharge a single calcium action potential. These action potentials, shown truncated in this and other chart records, were followed by a calcium-activated potassium a.h.p. *A*1, application of NA (10 $\mu$M for 5 min) markedly decreased the amplitude of the a.h.p. *A*2, application of phentolamine (10 $\mu$M for 12 min) had no action on the a.h.p. by itself, and failed to prevent an abolition of the a.h.p. when NA (10 $\mu$M for 3 min) was reapplied in the presence of this antagonist. *A*3, application of propranolol (10 $\mu$M for 10 min) had no effect on the a.h.p. by itself, but largely prevented the reduction of the a.h.p. when NA was reapplied (10 $\mu$M for 3 min). The membrane potential of this cell was −60 mV. *B*, records from another pyramidal cell showing that a low dose (0·3 $\mu$M) of propranolol prevented both the noradrenergic reduction in the a.h.p. and also the NA-induced depolarization of the resting membrane potential (insets). NA applications (1 $\mu$M) were begun at the time indicated by the arrows. The membrane potential of this cell was −64 mV.

of −40 mV. Periodic hyperpolarizing command voltages were applied which caused a slow inward relaxation in the current record. This relaxation, which was presumably due to the voltage-dependent inactivation of the M-current, was not reduced by NA application (10 $\mu$M, $n = 6$) although NA did cause an inward shift in holding current

along with an increase in input resistance. Application of the muscarinic agonist carbachol (40 $\mu$M) caused a further inward shift in the holding current and blocked the voltage-dependent current relaxation, confirming that it was due to the M-current (Adams *et al.* 1982). Thus, since NA does not reduce the M-current, it seems unlikely that blockade of this current underlies the depolarizing action of NA. Another conductance which could possibly underlie this action of NA is the calcium-activated potassium conductance.

*Effects of NA on calcium-activated potassium a.h.p.s*

A slow a.h.p. due to calcium-activated potassium conductance ($G_{K(Ca)}$) follows depolarizing stimuli in pyramidal cells (Alger & Nicoll, 1980*a*; Hotson & Prince, 1980; Schwartzkroin & Stafstrom, 1980; Gustafsson & Wigström, 1981; Madison & Nicoll, 1984*a*). NA application reduced the size of the a.h.p. (Madison & Nicoll, 1982; Haas & Konnerth, 1983; Fig. 3) as well as the underlying outward current recorded under voltage clamp (Lancaster & Adams, 1984). This effect was seen at concentrations of NA as low as 30 nM and often occurred without any change in the resting membrane potential. A concentration of 10 $\mu$M-NA caused a mean reduction in a.h.p. amplitude of 93 % ($\pm$14·4, S.D., $n = 26$). In addition to reducing the amplitude of the a.h.p., NA also accelerated the rate of decay of the a.h.p. This effect of NA was quite consistent; for a reduction in a.h.p. amplitude of 50 %, NA caused a decrease in the time constant of the a.h.p. decay from a control average of 2·5 to 1·3 s ($n = 8$).

To characterize further the mechanism by which NA reduced the a.h.p., experiments were performed to determine the receptor subtype which mediated this effect. In Fig. 3*A*, a pyramidal cell, bathed in 1 $\mu$M of the sodium channel blocker, TTX, and 5 mM of the potassium channel antagonist, TEA, was stimulated by a brief duration current pulse which caused the cell to fire a calcium action potential (cf. Schwartzkroin & Slawsky, 1977). This calcium action potential in turn elicited a calcium-activated potassium a.h.p. Addition of 10 $\mu$M-NA to the bathing medium caused a large reduction in the amplitude of this a.h.p. (Fig. 3*A*1). The $\alpha$-receptor antagonist phentolamine failed to prevent this action of NA (Fig. 3*A*2), even at concentrations as high as 20 $\mu$M. The $\beta$-receptor antagonist, propranolol (10 $\mu$M) markedly attenuated this action of NA (Fig. 3*A*3). Doses of propranolol as low as 0·3 $\mu$M could abolish both the NA-induced blockade of a.h.p.s and the depolarization of the resting membrane potential (Fig. 3*B*).

$\beta$-Adrenergic agonists were also effective in reducing the a.h.p. Comparative concentration–response relationships for the agonists isoprenaline, NA and adrenaline were all recorded from the same pyramidal cell (Fig. 4) in six different experiments. Isoprenaline was found to be effective at a concentration nearly ten times lower than NA, while adrenaline was roughly equipotent with NA (Fig. 4). Phenylephrine (10 $\mu$M, $n = 9$) and clonidine (10–100 $\mu$M, $n = 3$) were without effect on the after-hyperpolarization (not shown). These results taken together with the results of the antagonist experiments, indicate that NA reduced evoked calcium-activated potassium conductance by activating a $\beta$-adrenergic receptor.

We have further characterized the receptor type responsible for the NA-induced blockade of $G_{K(Ca)}$ by using $\beta_1$- and $\beta_2$-selective agonists and antagonists. Atenolol, which selectively antagonizes the $\beta_1$-receptor (Barratt, 1977), and butoxamine, which

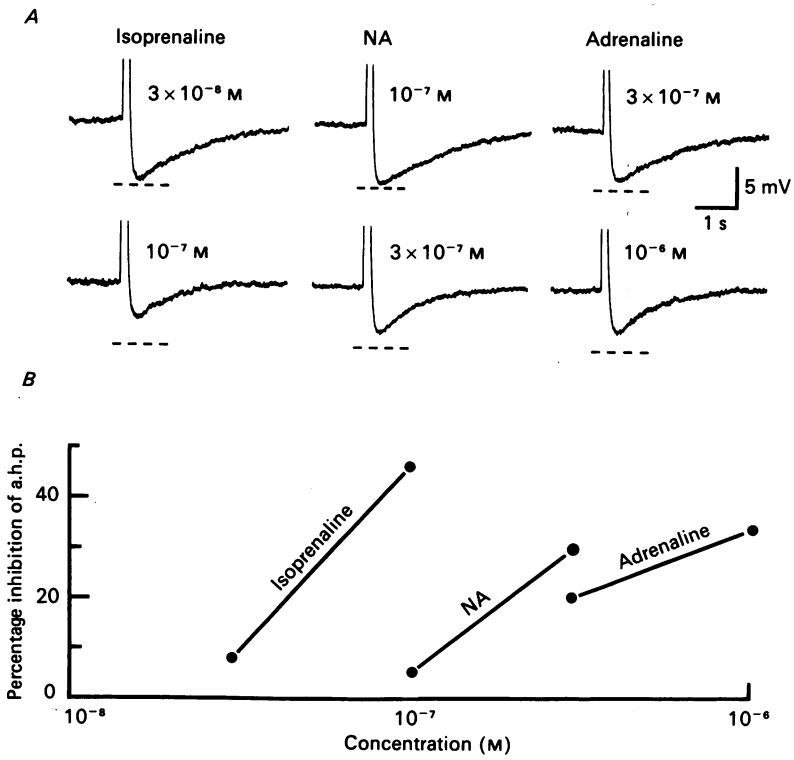


Fig. 4. $\beta$-Adrenergic agonists reduce calcium-activated potassium a.h.p.s. All responses shown here were recorded from a single CA1 pyramidal neurone bathed in TTX (1 $\mu$M) and TEA (5 mM). *A*, chart records showing the a.h.p. which follows a current-evoked calcium action potential. Three agonists, isoprenaline, NA and adrenaline were applied to the cell by bath application. A.h.p.s were measured after the effect of each dose of agonist had stabilized. The dashed line beneath each trace indicates the amplitude of the control response before the agonist was applied. *B*, semilog plot of the single-cell concentration–response relationship derived from the data in *A*. The membrane potential of this cell was −58 mV.

blocks $\beta_2$-receptors (Levy, 1966), were used. In the experiment illustrated in Fig. 5, application of NA (10 $\mu$M) caused a large decrease in the amplitude of the a.h.p. (Fig. 5*A*). Noradrenaline was then washed from the bath until the a.h.p. recovered. Application of 1 $\mu$M of the $\beta_1$-antagonist atenolol caused no change in the a.h.p. by itself, but prevented the reduction in the a.h.p. when NA was reapplied (Fig. 5*B*, $n = 5$). The blockade of the receptor by atenolol was reversible (Fig. 5*C*). The $\beta_2$-antagonist butoxamine (1 $\mu$M) was then applied, and this failed to prevent the action of NA (Fig. 5*D*, $n = 3$).

The noradrenergic receptor-sensitivity of the a.h.p. could also be differentiated on the basis of the actions of $\beta_1$- and $\beta_2$-selective agonists. The $\beta_2$-agonist salbutamol (Brittain, Farmer, Jack, Martin & Simpson, 1968; Cullum, Farmer, Jack & Levy, 1969), when applied to the preparation, had no effect on the a.h.p. (Fig. 6*A*, $n = 2$) at concentrations of up to 100 $\mu$M (highest tested). On the other hand, the $\beta_1$-selective

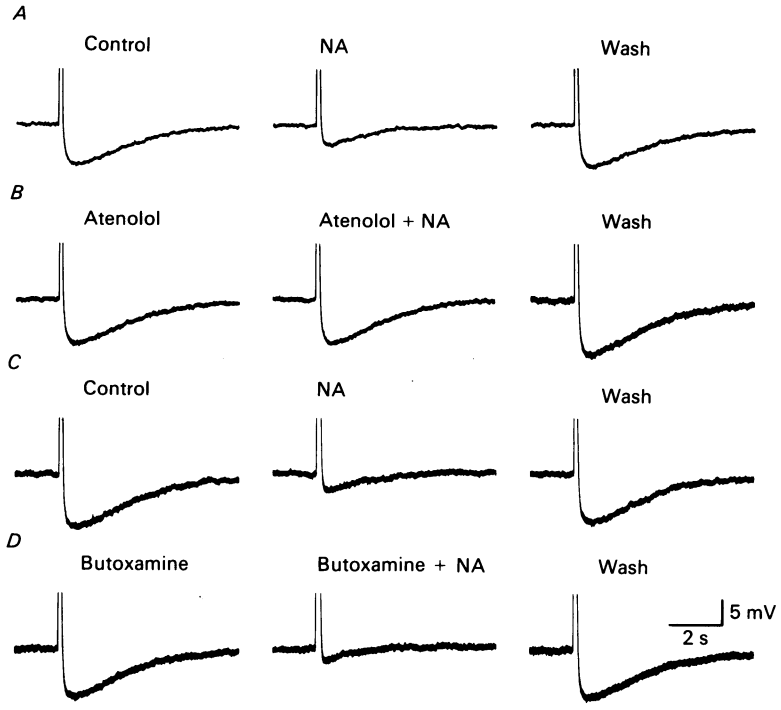


Fig. 5. The effects of $\beta_1$- and $\beta_2$-selective antagonists on the action of NA. All records in this Figure were taken from the same pyramidal cell bathed in TTX (1 $\mu$M) and TEA (5 mM). *A*, application of NA (1 $\mu$M for 6 min) reversibly reduced the amplitude of the a.h.p. following a current evoked calcium action potential. *B*, application of the selective $\beta_1$-antagonist atenolol (1 $\mu$M for 31 min) prevented the reduction of the a.h.p. following reapplication of NA (1 $\mu$M for 5 min). *C*, after washing NA and atenolol from the bath (for 56 min) reapplication of NA (1 $\mu$M for 6 min) again caused a reduction of the a.h.p. *D*, application of the selective $\beta_2$-antagonist butoxamine (1 $\mu$M for 32 min) did not prevent the reduction in the a.h.p. following reapplication of NA (1 $\mu$M for 6 min). The membrane potential of this cell was −64 mV.

agonist, dobutamine (100 $\mu$M) (Tuttle & Mills, 1975), was effective in reducing the a.h.p. (Fig. 6*B*, $n = 3$). These experiments indicate that NA reduced the a.h.p. through an action on a $\beta_1$-receptor.

*Mechanism of noradrenergic blockade of calcium-activated potassium a.h.p.s*

In the sympathetic ganglion (Horn & McAfee, 1980; Galvan & Adams, 1982), dorsal root ganglion cells (Dunlap & Fischbach, 1981), and locus coeruleus neurones (Williams & North, 1985), NA reduced calcium action potentials or the inward calcium current observed under voltage clamp. This effect, in sympathetic ganglion cells was mediated by an $\alpha$-adrenergic receptor and resulted in reductions in the calcium-activated potassium a.h.p. (Horn & McAfee, 1980). To test for possible NA effects on calcium entry into pyramidal cells, we have compared the effect of NA on calcium action potentials with that of cadmium, an agent which blocks calcium channels. Shown in Fig. 7 are three records from the same cell selected to illustrate the effect

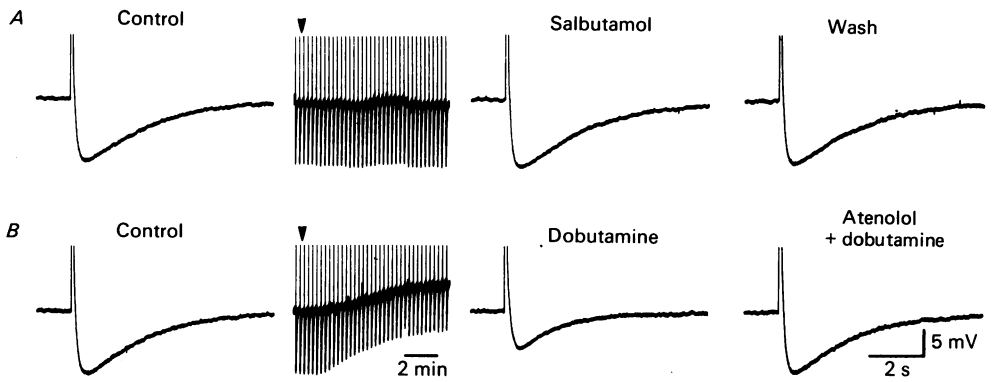


Fig. 6. Effect of $\beta_1$- and $\beta_2$-selective agonists on the a.h.p. All records in this Figure were taken from the same pyramidal cell bathed in TTX (1 $\mu$M) and TEA (5 mM). *A*, the a.h.p. following a current-evoked calcium action potential was not reduced by application of 100 $\mu$M of the $\beta_2$-selective agonist salbutamol (12 min). Salbutamol had no effect on the resting membrane potential (inset). *B*, after washing salbutamol from the recording chamber, application of the $\beta_1$-selective agonist dobutamine (100 $\mu$M for 10 min) reduced the amplitude of the a.h.p. and depolarized the cell (inset). Addition of the $\beta_1$-selective antagonist atenolol (10 $\mu$M for 11 min, with dobutamine still present) reversed the actions of dobutamine. The membrane potential of this cell was −63 mV.

of NA on the calcium action potential and the corresponding a.h.p. When NA (10 $\mu$M) was applied to pyramidal cells, bathed in TTX (1 $\mu$M) and TEA (5 $\mu$M), the a.h.p. was reduced without any reduction in the calcium action potential (Fig. 7*A*). NA was then washed from the recording chamber until the a.h.p. returned to its control amplitude. Cadmium (100 $\mu$M) was then applied to the preparation (Fig. 7*B*). Application of cadmium caused a reduction in the a.h.p. but, unlike NA, also reduced the amplitude of the calcium action potential *pari passu*. In Fig. 7*C*, the areas of the calcium action potentials are plotted against the areas of the corresponding after-hyperpolarizations. With cadmium, the reductions in these two parameters were closely correlated ($r^2 = 0{\cdot}95$) while with NA, there was little correlation ($r^2 = 0{\cdot}12$) as determined by the least-squares regression method. These results suggest that NA and cadmium reduce the a.h.p. through different mechanisms, i.e. that NA does not block calcium channels and must be acting at some step after calcium entry to reduce the a.h.p. This conclusion is supported by the findings that NA did not depress the rate of rise of the calcium action potential, nor did it decrease calcium action potentials recorded after blockade of potassium channels with intracellular caesium injection and 30 mM-extracellular TEA (not shown).

As a further measure of whether NA can reduce calcium entry into pyramidal cells, we have measured inward calcium and barium currents using a single-electrode voltage clamp (cf. Brown & Griffith, 1983*b*). Cells were recorded with caesium-chloride-filled micro-electrodes and bathed in medium containing 0·5 $\mu$M-TTX, 30 mM-TEA and high magnesium (15 mM). To examine current carried by calcium ions, calcium in the medium was reduced to 1 mM, and for barium currents, calcium was removed and replaced with 1 mM-barium chloride. These conditions were designed to reduce outward currents so that inward currents could be examined in

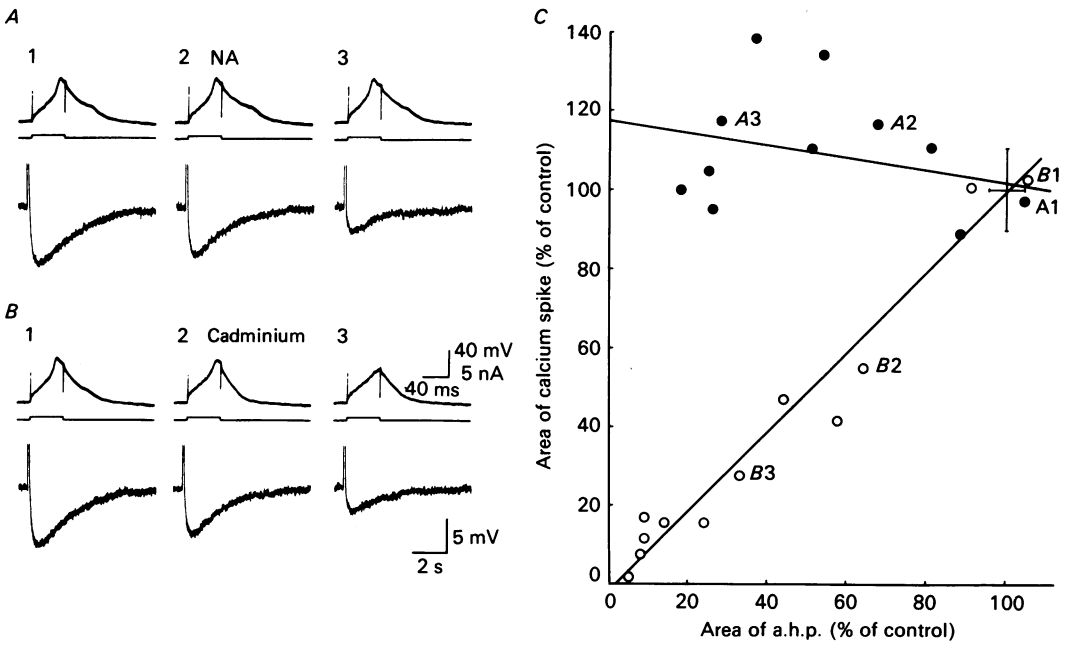


Fig. 7. Comparison of the actions of NA and cadmium on the a.h.p. and calcium action potential. All records in this Figure were taken from the same pyramidal cell bathed in TTX (1 $\mu$M) and TEA (5 mM). *A*1, photographic records of calcium action potentials generated in response to a short-duration depolarizing current pulse. The current monitor trace is positioned below the voltage trace. *A*2, the a.h.p.s shown on chart records correspond to the action potential above. Application of NA (10 $\mu$M) caused a decline in the amplitude of the a.h.p. (records 1–3 are in sequential order from left to right), but did not reduce the accompanying calcium action potential. *B*, after the a.h.p. had returned to control amplitude following wash-out of NA from the recording chamber, the calcium-channel blocker cadmium (100 $\mu$M) was added to the bathing medium and caused a sequential decline in both the calcium action potential and the a.h.p. *C*, graph showing the relationship between the area of the calcium action potential and the area of the a.h.p., during the application of NA (filled circles), and cadmium (open circles). Note that during NA application there is little relationship between the calcium action potential area and the area of the a.h.p. ($r^2 = 0{\cdot}12$ by linear regression) while during cadmium application there is a high degree of correlation between reduction in the area of the calcium action potential and reduction in the size of the a.h.p. ($r^2 = 0{\cdot}95$). The error bars indicate the standard deviations for the areas of the a.h.p. and calcium action potential in control conditions in this cell ($n = 15$). The membrane potential of this cell was −56 mV.

relative isolation. Inward currents were produced by making depolarizing steps from holding potentials between −30 and −40 mV. These inward currents were not reduced by NA application (10 $\mu$M, $n = 7$, Fig. 8), and often were actually slightly increased in NA. Application of cadmium (100 $\mu$M) on the other hand, caused an almost complete abolition of these currents ($n = 7$), suggesting that they were indeed carried through calcium channels. Under normal recording conditions, this concentration of NA (10 $\mu$M) caused better than a 90% reduction in the amplitude of the a.h.p. Thus, it is very likely that a reduction in calcium entry cannot account for the reduction of the a.h.p. caused by NA in pyramidal cells, and that NA must

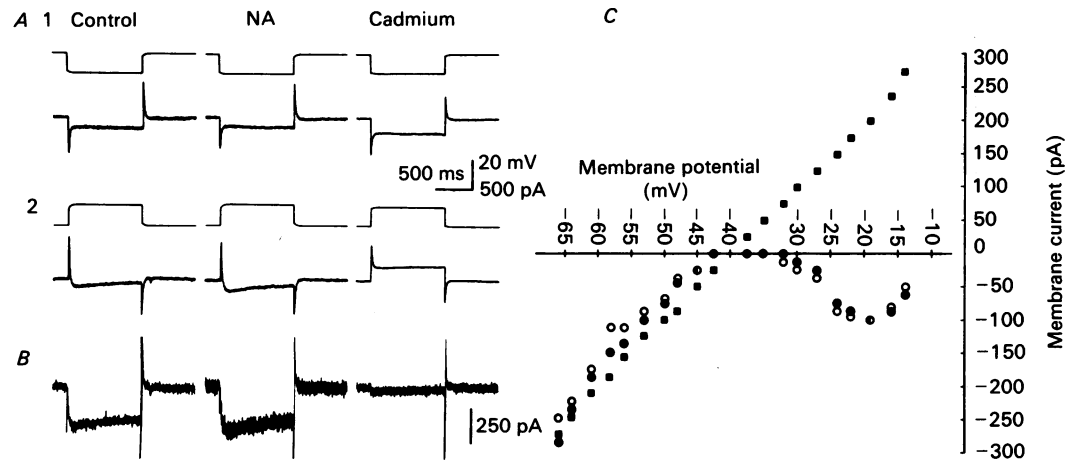


Fig. 8. NA does not reduce inward barium currents carried by calcium channels. All responses in this Figure were recorded from the same pyramidal cell. Traces shown were recorded on a single-electrode voltage clamp and are responses to depolarizing and hyperpolarizing voltage commands from a holding potential of −40 mV. *A*, membrane voltage is shown here above the current trace. The responses of the cell to hyperpolarizing (*A*1) and depolarizing (*A*2) steps were recorded in control conditions, after 10 min in 10 μM-NA, and 14 min after wash-out of NA and application of 100 μM-cadmium. Note that NA application did not reduce the inward barium current elicited in response to depolarizing steps, but that this current was markedly reduced by cadmium. *B*, digital plotter records showing the inward current after subtraction of the leakage current (i.e. the reversed current from the hyperpolarizing step was subtracted from the depolarization-induced current). The apparent increase in current noise in the subtracted record during NA application was produced by a subtraction artifact and was not a consistent finding. *C*, current–voltage plot of the unsubtracted peak membrane current elicited by a range of voltage steps from −40 mV in control (filled circles), in NA (open circles) and in cadmium (squares).

exert its action on the calcium-activated potassium conductance at some step after calcium entry.

One possible mechanism by which NA could block the a.h.p. is by increasing the intracellular sequestration of calcium so that the rise in intracellular calcium following an action potential is prevented. We have tested this possibility by comparing the effects of intracellular injection of the calcium chelator, EGTA, to the effects of maximal β-receptor stimulation on calcium action potentials. With high concentrations of β-agonists there was often some broadening of the base of the calcium action potentials (see Fig. 3*A*) and some cells fired repetitive calcium action potentials (Fig. 9*A*). However, the repolarizing phase of the calcium action potential was not altered (Fig. 9*A*). On the other hand, injection of ETGA (0·2 M in 2 M-$KMeSO_4$), unlike β-receptor stimulation, always delayed repolarization and caused a progressive lengthening of the calcium action potential (Fig. 9*B*) ($n = 7$) which typically lasted hundreds of milliseconds in this condition. This lengthening occurred even before EGTA had reduced the a.h.p. to the same extent as did NA. This suggests that calcium binding by EGTA exerts some additional effect on the cell compared to β-receptor stimulation.

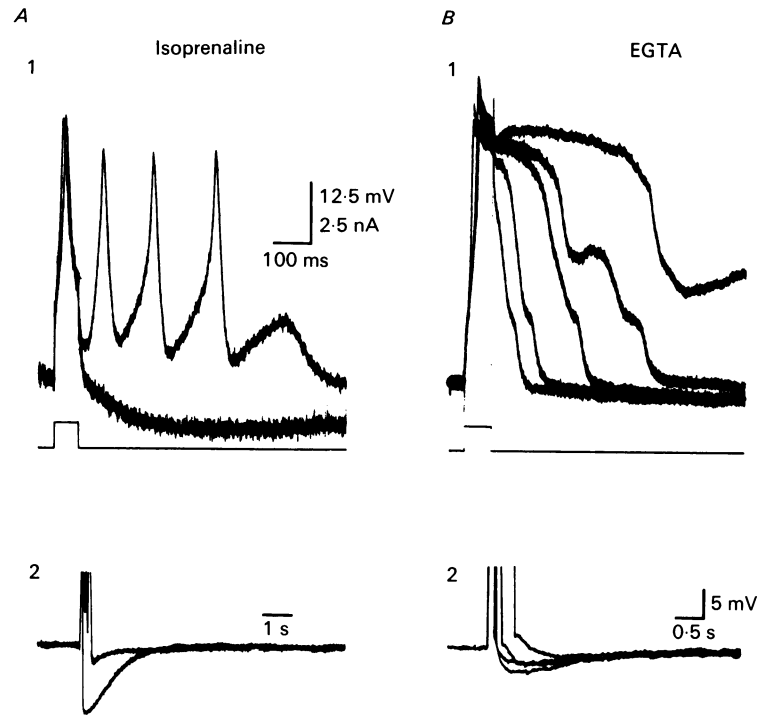


Fig. 9. $\beta$-Receptor stimulation and intracellular injection of EGTA have dissimilar actions on calcium action potentials. *A*, records from a pyramidal cell bathed in 1 $\mu$M-TTX and 5 mM-TEA. *A*1, superimposed digital plotter records showing calcium action potentials elicited by a short duration current pulse in control and after 9 min in 200 $\mu$M-isoprenaline (bath application). *A*2, superimposed tracings of a.h.p.s corresponding to the records in *A*1. The membrane potential of this cell was $-55$ mV. *B*, records from another pyramidal cell bathed in the same medium as the cell in *A*, and impaled with an EGTA-filled micro-electrode (0·2 M-EGTA in 2 M-$KMeSO_4$). *B*1, superimposed digital plotter records of a current-evoked calcium action potential taken from the cell 30 s, 1·5 min, 6 min, 9 min, and 19 min after impalement. *B*2, superimposed tracings of a.h.p.s corresponding to the first, second, third and fifth action potentials shown in *B*1. The membrane potential of this cell was $-58$ mV. Note that even in this extreme case of $\beta$-receptor action the cell repolarizes between each calcium action potential; with EGTA-filled cells a single action potential with a long plateau phase was produced.

*Functional consequences of NA action*

It has previously been reported that NA increases the excitatory action of the putative neurotransmitter, glutamate, in a number of regions in the central nervous system (Woodward *et al.* 1979; Rogawski & Aghajanian, 1980; Segal, 1982). Fig. 10 shows the response of a pyramidal cell to a pulse of ionophoretically applied glutamate. Glutamate application (50 nA) resulted in a depolarization of the membrane potential which elicited a train of action potentials from the pyramidal cell (Fig. 10*A*1). While NA (5 $\mu$M) had little effect on the size of the depolarization caused by glutamate, the number of action potentials elicited by the depolarization was dramatically increased ($n = 9$). The glutamate activation was followed by an a.h.p.

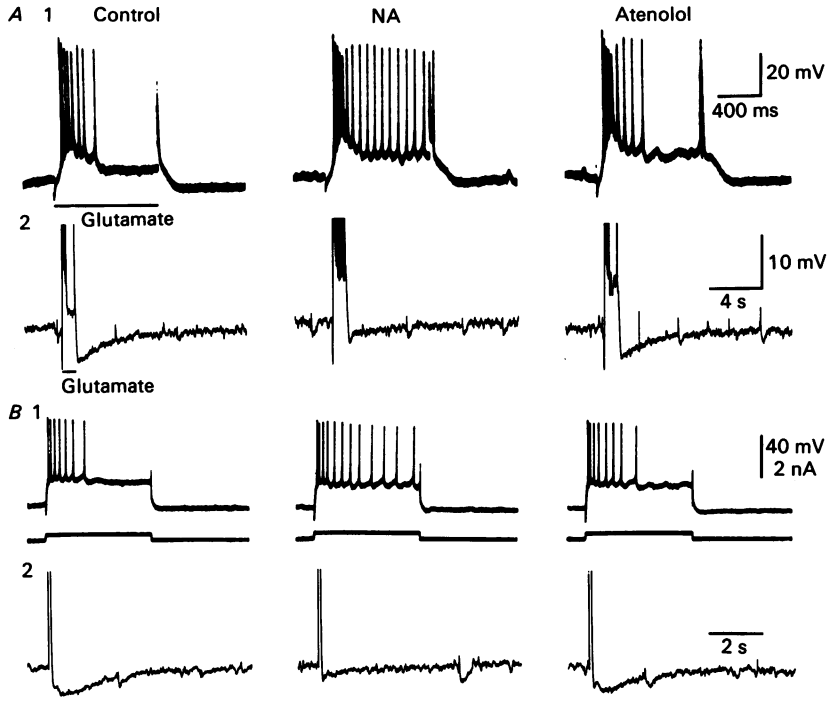


Fig. 10. NA reduces accommodation by reducing calcium-activated potassium a.h.p.s. All records shown in this Figure were taken from the same pyramidal cell bathed in normal medium. Responses shown in each part of the Figure were recorded concurrently. *A*1, photographic records of the cell's response to ionophoretically applied (50 nA) glutamate. In control conditions application of glutamate produced a depolarization of the membrane potential which elicited a short train of action potentials. After application of 5 $\mu$M-NA (for 7 min), an identical glutamate application produced a higher frequency and longer duration train of action potentials. *A*2, chart records of the same responses shown in *A*1, at a slower speed, to show the a.h.p. that follows glutamate activation of the cell. Note that this a.h.p. is reduced by NA during the time when action potential discharge is heightened. *B*, photographic records of the same cell's response to depolarizing current pulses of long (*B*1) and short (*B*2) duration. In *B*1 the current trace is positioned below the voltage trace. Note that NA application reduced the a.h.p. (*B*2) and decreased action potential accommodation (*B*1). All actions of NA shown here were reversed by application of the $\beta_1$ antagonist atenolol (3 $\mu$M for 13 min). The membrane potential of this cell was $-57$ mV.

due to calcium-activated potassium conductance. This a.h.p. was reduced during the time when the cell's responsiveness to glutamate was increased by NA (Fig. 10*A*2). The increase in responsiveness caused by NA application was not confined to glutamate excitations. In this same cell, NA application also reduced the accommodation of cell discharge evoked by long duration depolarizing current pulses passed through the recording electrode (Fig. 10*B*1). A previous study has shown that spike frequency accommodation is caused primarily by calcium-activated potassium conductance in these cells (Madison & Nicoll, 1984*a*). NA application reduced the calcium-activated potassium conductance associated with this accommodation (Fig. 10*B*2, $n = 35$). When stimulated with large amplitude, 650 ms long, depolariz-

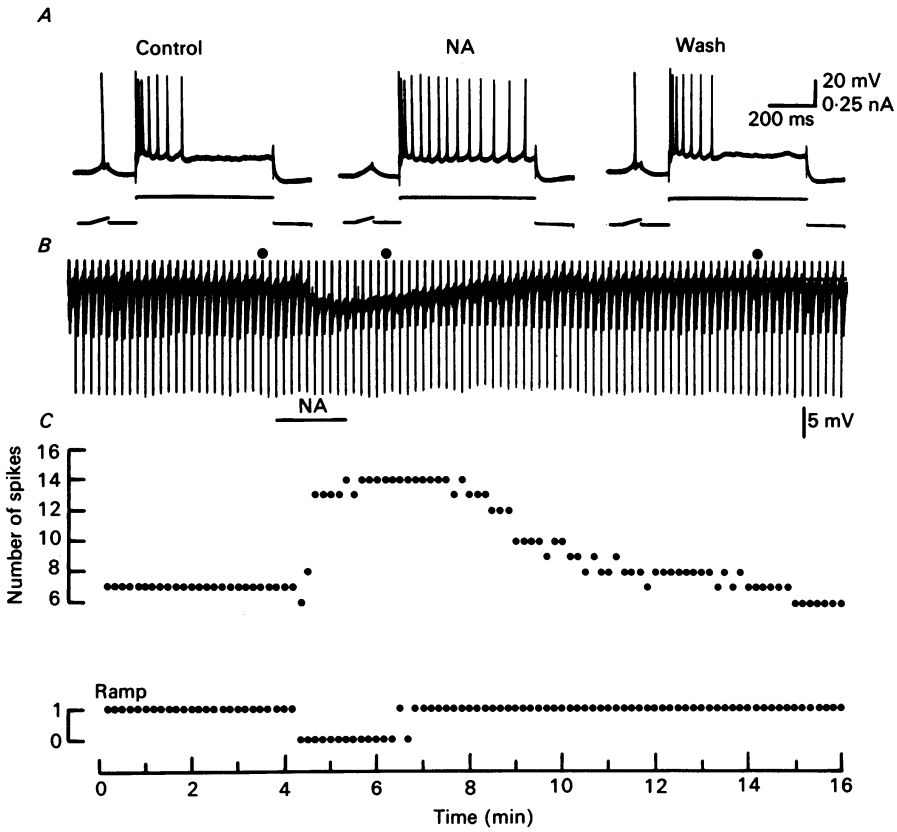


Fig. 11. NA increases the signal-to-noise ratio of hippocampal pyramidal cells. All records shown were taken from the same pyramidal cell bathed in normal medium. *A*, film records of the response of the pyramidal cell to a depolarizing current stimulus consisting of a short ramp depolarization, just threshold to elicit a single action potential, followed by a large amplitude, long duration depolarizing current pulse. Records shown were recorded in control, 4 min after addition of 10 $\mu$M-NA to the superfusing medium, and 15 min after the NA was washed from the recording chamber. *B*, continuous chart record of the membrane potential of the pyramidal cell. The responses in *A* were taken at the points marked by the filled circles. Note that there are two types of hyperpolarizing deflexions in this record. The larger of these deflexions are constant current hyperpolarizing pulses, used to measure the membrane input resistance. The smaller deflexions are a.h.p.s elicited by the long depolarizing stimulus. These depolarizations are seen as truncated upward deflexions in the record. *C*, plot of the number of spikes elicited over the course of the experiment by the long depolarizing stimulus (upper graph) and by the ramp stimulus (lower graph). Note that application of NA caused a hyperpolarization of the membrane potential, with an increase in conductance, which was large enough to inhibit the response of the cell to the small ramp stimulus, while the response to the large stimulus was enhanced. The membrane potential of this cell was $-55$ mV.

ing current pulses in control conditions, pyramidal cells fired $6{\cdot}8 \pm 1{\cdot}7$ action potentials, and $16{\cdot}8 \pm 2{\cdot}7$ action potentials in the presence of NA ($n = 14$). All of these actions of NA could be antagonized by application of the $\beta_1$-antagonist, atenolol (3 $\mu$M) (Fig. 10, right column).

*Interactions between different noradrenergic actions*

Interactions between the NA-induced blockade of calcium-activated potassium a.h.p.s and effects of NA on resting membrane potential could often be observed. Specifically, an interaction was recorded between the blockade of the a.h.p. and membrane potential hyperpolarizations caused by NA. In Fig. 11, a pyramidal neurone was stimulated with a small ramp of depolarizing current which was just above threshold for eliciting a single action potential. This ramp stimulus was followed by a large, 600 ms long, step depolarizing pulse which elicited a characteristic train of action potentials followed by a silent period (cf. Lanthorn, Storm & Andersen, 1984; Madison & Nicoll, 1984*a*). Application of NA (10 $\mu$M) caused a hyperpolarization of the resting membrane potential which was of sufficient magnitude to inhibit the response of the cell to the near threshold stimulus. At the same time, NA caused a reduction in the calcium-activated potassium a.h.p. which resulted in an enhancement of the cell's response to the large depolarizing stimulus. Thus, the interaction between the membrane potential hyperpolarization and the $\beta$-receptor mediated reduction in $G_{K(Ca)}$ is capable of causing an increase in the 'signal-to-noise ratio' of the neurone such that responses to small, near threshold stimuli are inhibited, while the responses to stimuli large enough to overcome this inhibition are enhanced.

## DISCUSSION

NA has been shown here to have two types of direct actions on CA1 pyramidal neurones: effects on the resting membrane potential and blockade of calcium-activated potassium a.h.p.s.

The hyperpolarizing action of NA in the present study was small and variable thus prohibiting a thorough analysis of the receptor type involved and the underlying ionic mechanism. Nevertheless, the pharmacology of this hyperpolarization was most consistent with that of an $\alpha$-adrenergic receptor, since it was blocked by phentolamine, and because the $\alpha$-agonists phenylephrine and clonidine could also produce hyperpolarizations. Previous studies in the hippocampus have reported inhibition of spontaneous firing recorded extracellularly (Segal & Bloom, 1974*a*, *b*), and intracellulary recorded hyperpolarizations (Herrling, 1981, Langmoen *et al.* 1981; Segal, 1981). These effects of NA have most often been ascribed to the action of a $\beta$-receptor (Segal & Bloom, 1974*a*, *b*; Segal, 1981, but see Mueller *et al.* 1982). Our findings are more consistent with the action of an $\alpha$-receptor. However, it should be noted that high doses of the $\alpha$-antagonist phentolamine were required to block this hyperpolarization, and therefore the receptor type for this noradrenergic response has not been fully characterized. $\alpha$-Receptor-mediated hyperpolarizations have been reported in mammalian sympathetic (Brown & Caulfield, 1979) and parasympathetic neurones (Nakamura, Yoshimura, Shinnick-Gallagher, Gallagher & Akasu, 1984) and in locus coeruleus neurones (Egan, Henderson, North & Williams, 1983).

Extracellularly recorded excitatory effects, and an intracellularly recorded depolarizing action of NA, have been observed by others in hippocampal slices (Mueller *et al.* 1981, 1982; Gruol & Siggins, 1982; Otmakhov & Bragin, 1982). The depolarizing action of NA, reported here is associated with an increase in the input resistance of

the cell and is mediated by a $\beta$-receptor since it is attenuated by propranolol, and can be reproduced by the $\beta$-agonist, isoprenaline. Furthermore, this depolarizing action of NA can be mimicked by agents which increase the intracellular concentration of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (Madison & Nicoll, 1986), as would be expected for a response mediated by a $\beta$-receptor. $\beta$-Receptor-mediated depolarizations have also been reported in the superior cervical ganglion (De Groat & Volle, 1966; Haefely, 1969; Brown & Dunn, 1983).

Both of these responses, the hyperpolarization and the depolarization, appear to be, at least in part, direct actions on the pyramidal cell as they both persist in the presence of TTX, which would block any action potential-dependent indirect effects. Since both of these effects occur in cells in which the chloride gradient had been reversed, which would reverse the polarity of chloride-mediated potentials, it is likely that both the hyperpolarizing and depolarizing responses to NA are due, at least in part, to changes in potassium conductance. It should be noted however, that NA causes an increase in the frequency of chloride-mediated spontaneous inhibitory synaptic potentials (Madison & Nicoll, 1984*b*; see Alger & Nicoll, 1980*b*) which could result in a small hyperpolarizing action on pyramidal cells. Such an action could possibly account for an indirect hyperpolarizing action of NA.

As the depolarizing response is accompanied by an increase in input resistance, and is not reversed by chloride injection, it follows that it is most likely to be due to a decrease in a resting potassium conductance. A mechanism that could possibly account for the depolarization is a blockade of the voltage-sensitive M-current. An M-current has been described in hippocampal pyramidal cells (Halliwell & Adams, 1982), but this current is not reduced by NA. Another possibility is that NA may depolarize pyramidal cells by blocking a small, and variably present, resting calcium-activated potassium conductance ($G_{K(Ca)}$). Such an hypothesis is supported by the finding that NA blocks the calcium-activated potassium a.h.p. evoked by a series of action potentials. This action of NA is also mediated by the action of a $\beta$-adrenergic receptor. However, since the calcium channel blocker, cadmium, does not mimic the depolarizing action of NA, it is unlikely that NA blocks a resting $G_{K(Ca)}$ resulting from tonic influx of calcium across the cell membrane, as has been suggested for the action of serotonin in myenteric plexus neurones (Grafe, Mayer & Wood, 1980). NA could possibly reduce a tonic $G_{K(Ca)}$ by reducing free calcium availability intracellularly (cf. Tsien, 1977) or by blocking the potassium channel directly.

In spite of the fact that NA causes changes in the resting membrane potential of pyramidal cells, these changes are very much smaller than membrane potential changes caused by NA in other central nervous system structures (cf. Egan *et al.* 1983). The small size of these effects and their extreme variability makes it seem unlikely that these are the major actions of NA on pyramidal cells. Indeed, a far more robust and reproducible action of NA in the hippocampus is the blockade of calcium-activated potassium a.h.p.s.

NA reduces the calcium-activated potassium a.h.p. through an action on $\beta_1$-receptors. This conclusion is based on the following evidence: (i), the rank order of potency of the agonists: isoprenaline > NA $\geqslant$ adrenaline; corresponds to that of a $\beta$-receptor; (ii), the $\beta$-antagonist, propranolol, and the $\beta_1$-antagonist, atenolol, both prevented the NA-induced reduction in the a.h.p., while the $\beta_2$-antagonist, butoxamine,

had no such effect; (iii), the $\beta_1$-selective-agonist, dobutamine, caused a reduction in the a.h.p. while the $\beta_2$-selective-agonist, salbutamol, had no such effect; (iv), $\alpha$-agonists and antagonists were without effect on the a.h.p.

The mechanism for the NA-induced blockade of calcium-activated potassium a.h.p.s is quite different from that described in other types of neurones. Previous experiments, in peripheral ganglion cells (Horn & McAfee, 1980; Dunlap & Fischbach, 1981; Galvan & Adams, 1982), and in locus coeruleus neurones (Williams & North, 1985), have suggested that NA can reduce calcium currents. This does not appear to be the case in the hippocampal pyramidal cells as our experiments have consistently failed to demonstrate an effect of NA on calcium action potentials or calcium currents. In fact, in many cells, the size of this inward current was often increased slightly during NA application. It has been reported that a transient calcium current can be elicited in these cells by depolarizing steps from holding potentials more negative than those used in this study (Halliwell, 1983). We have not examined this current. However, since the size of the calcium action potential, which we have found to be an accurate monitor of the calcium responsible for generating the a.h.p. (see Fig. 7), is not reduced by NA, it seems unlikely that a selective action of NA on this transient calcium current could account for the a.h.p. depression.

If NA does not prevent calcium entry, then how does it block a.h.p.s? Two possibilities are that NA causes an increase in calcium sequestration, as occurs in the heart (cf. Tsien, 1977), or a blockade of the potassium channels responsible for a.h.p.s. In addition to decreasing the amplitude of the a.h.p., NA also consistently decreased its duration by speeding the rate of decay from peak (cf. North & Tokimasa, 1983; for a similar effect of acetylcholine on the a.h.p. of myenteric neurones). Although this might suggest that NA reduces a.h.p.s by stimulating the intracellular sequestration of free calcium, this effect in pyramidal cells is always contaminated with a substantial decrease in a.h.p. amplitude. Thus, increased sequestration of calcium may not be the primary mechanism involved in a.h.p. blockade. A comparison of the effects of injecting the calcium chelator, ethyleneglycol-bis-($\beta$-aminoethylether)*N*,*N*′ tetraacetic acid (EGTA), to the effects of $\beta$-receptor stimulation and 8-bromo cyclic AMP (Madison & Nicoll, 1986) on the calcium action potential also suggests that calcium sequestration may not be involved in blocking the a.h.p. Injecting EGTA caused a progressive lengthening of the calcium action potential so that a plateau lasting hundreds of milliseconds was produced. This lengthening occurred at a time when the a.h.p. was larger than that recorded after the maximal action of $\beta$-agonists, suggesting that EGTA in addition to blocking the a.h.p. also affected repolarization of the calcium action potential. While $\beta$-receptor stimulation could, in many instances, slightly prolong the base of the calcium action potential and induce repetitive firing, the repolarizing phase of the action potential was immune to $\beta$-receptor stimulation. Thus, preventing the rise in intracellular calcium with EGTA has an effect on action potential repolarization which is not shared by $\beta$-receptor stimulation. Removal of a calcium-mediated inactivation of calcium current (Tillotson, 1979) by EGTA could explain the difference, or alternatively there could be two calcium-activated potassium currents; only one of which is involved in spike repolarization and which is unaffected by $\beta$-receptor stimulation. Recent evidence

obtained in bull-frog sympathetic ganglia indicates that two quite distinct calcium-activated potassium currents, termed $I_C$ and $I_{a.h.p.}$, do exist (Pennefather, Lancaster, Adams & Nicoll, 1985). Although the distinction is less clear in pyramidal cells, currents which resemble $I_C$ (Brown & Griffith, 1983) and $I_{a.h.p.}$ (Lancaster & Adams, 1984) have been described.

The consequences of the NA-induced blockade of calcium-activated a.h.p.s on pyramidal cell physiology are quite striking. This blockade greatly reduces accommodation of action potential discharge in response to a depolarizing stimulus. By this action NA augments the response of the cell to excitatory stimuli. Previous reports have suggested that such an augmentation may occur because NA makes neurones more sensitive to excitatory neurotransmitters (Woodward *et al.* 1979; Segal, 1982). Our results suggest that the mechanism of this facilitation is more general in that NA makes the cell more responsive to any excitatory stimulus by blocking the cell's intrinsic inhibitory $G_{K(Ca)}$. This action is similar in many ways to excitatory effects reported in other systems such as serotonergic excitation of myenteric neurones (Grafe *et al.* 1980) or activation of a presynaptic serotonergic neurone on a follower neurone in *Helix* (Cottrell, 1982).

It is of interest to speculate on the functional significance of these actions of NA. While some of the actions of NA described in this report, such as the hyperpolarization of the resting membrane potential, are like those of conventional neurotransmitters, the NA-induced reduction of the calcium-activated potassium a.h.p., which can occur without any accompanying changes in resting potential, is quite different. This action is unlike classical neurotransmitter actions in that, by itself, it has little effect on the neurone at rest, but rather, strongly alters the neurone's response to other excitatory inputs such as glutamate application.

Especially interesting in a functional sense, are the interactions which can occur between the different actions of NA reported here. An example of such an interaction can be seen between the hyperpolarizing action of NA and the NA-induced block of a.h.p.s. The hyperpolarization of resting membrane potential can inhibit the response of the neurones to small, just threshold excitatory stimuli, while the blockade of the a.h.p. enhances the cell's response to stimuli large enough to overcome the inhibition. This action of suppressing weak inputs while enhancing strong ones is consistent with the concept that NA may increase the 'signal-to-noise ratio' of the neurone (Woodward *et al.* 1979), or act as an 'enabling' system (Moore & Bloom, 1979).

Behavioural correlates to increased neuronal signal-to-noise ratios have been reported. Livingstone & Hubel (1981) found that when cats change from a state of sleep to one of wakefulness, the responsiveness of visual cortex neurones to visual stimuli is enhanced while spontaneous activity is unchanged or decreased. NA ionophoresis directly onto visual cortex neurones can produce similar changes in the responsiveness of these neurones to visual stimuli (Kasamatsu & Heggelund, 1982). It has also been reported that attentive fixation in monkeys is accompanied by a facilitation of the responses of parietal neurones to visual stimuli (Mountcastle, Andersen & Motter, 1981). NA would be an obvious candidate for mediating such effects, especially since wakefulness and attentiveness are conditions under which NA-containing locus coeruleus neurones are known to be active (Aston-Jones &

Bloom, 1981*a*, *b*). This type of modulation of neuronal responsiveness in behaving animals could occur perhaps through a mechanism similar to that described in this report.

One of the most striking aspects of the blockade of the a.h.p. is that NA is not the only neurotransmitter that has this action. Acetylcholine (Benardo & Prince, 1982; North & Tokimasa, 1983; Cole & Nicoll, 1984), serotonin (Grafe *et al.* 1980, Cottrell, 1982), histamine (Haas & Konnerth, 1983) and corticotropin releasing factor (Aldenhoff, Gruol, Rivier, Vale & Siggins, 1983) all decrease calcium-activated potassium a.h.p.s in various neuronal preparations. That such a diverse array of putative neurotransmitters can block this potassium conductance, and that this blockade has such a profound effect on the responsiveness of the affected neurone, implies strongly that the calcium-activated potassium channel plays a central role in the regulation of neuronal excitability, and indeed that it may serve as a final common path for the actions of many neurotransmitters.

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