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NORADRENERGIC AND SEROTONERGIC MODULATION OF A HYPERPOLARIZATION-ACTIVATED CATION CURRENT IN THALAMIC RELAY NEURONES

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SUMMARY

1. Modulation of the hyperpolarization-activated cation current, I_h, by noradrenaline (NA) and serotonin (5-HT) was examined in guinea-pig and cat medial and lateral geniculate relay neurones using the *in vitro* slice technique.

2. In the absence of pharmacological antagonists, local application of NA resulted in a slow depolarization and decrease in apparent input conductance, a response which was blocked by local or bath application of the α_1-adrenoceptor antagonist prazosin. Application of NA after pharmacological block of α_1- and α_2-adrenoceptors, or application of 5-HT in all conditions, induced a 1–3 mV slow depolarization which was associated with a pronounced increase in apparent input conductance. This response to NA and 5-HT persisted during blocked synaptic transmission and was present in both the guinea-pig and cat medial and lateral geniculate nuclei.

3. The increase in membrane conductance elicited by NA was mimicked by the β-specific agonist isoprenaline and blocked by the β-antagonists propranolol and atenolol, indicating that it is mediated by β-adrenoceptors. The response to 5-HT was blocked by the 5-HT_1 and 5-HT_2 antagonist methysergide, but not by the 5-HT_2 antagonist ritanserin. Applications of either the 5-HT_{1A} agonist ipsapirone or the partial agonist 8-hydroxy-dipropylaminotetralin (8-OHDPAT) were without effect.

4. Current *versus* voltage relationships obtained under voltage clamp revealed NA and 5-HT to cause a voltage-dependent inward shift at membrane potentials negative to approximately −60 mV. This response appeared to be shared by NA and 5-HT since maximal application of 5-HT greatly reduced or abolished the response to NA.

5. Application of NA and/or 5-HT during hyperpolarizing voltage steps in voltage clamp resulted in a marked increase in amplitude of the hyperpolarization-activated cation current, I_h. In addition, the rate of activation of I_h was strongly increased during activation of β-adrenoceptors.

6. The activation curve of the conductance underlying I_h (G_h) was shifted by 4–6 mV on the voltage axis with NA and/or 5-HT. The positive shift of G_h activation

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in the voltage domain resulted in an increase in the amplitude of G_h which is active at resting, and more hyperpolarized, membrane potentials. The subsequent increase in resting membrane conductance decreased the responsiveness of thalamic neurones to hyperpolarizations of all durations.

7. Local or bath application of caesium blocked both I_h and the increase in membrane conductance in response to NA and 5-HT. By contrast, barium blocked neither I_h nor the responses to NA and 5-HT.

8. The increase in I_h by NA and 5-HT may be mediated by an increase in adenylyl cyclase activity since I_h was enhanced by local application of the membrane-permeable cyclic AMP analogue, 8-bromo-cyclic AMP, the adenylyl cyclase stimulant, forskolin, and the phosphodiesterase inhibitor, 3-isobutyl-1-methyl-xanthine (IBMX). Applications of a forskolin analogue, 1,9-dideoxy-forskolin, which does not readily stimulate the production of cyclic AMP, had only minimal effects on I_h.

9. These results reveal a novel mechanism by which neurotransmitters may modulate the excitability of central neurones. In thalamic neurones, enhancement of I_h by activation of β- or serotonergic receptors results in a decrease in response to hyperpolarizing stimuli, a subsequent dampening of rhythmic burst discharges, and finally a slight facilitation of single-spike activity. In addition, activation of α_1-adrenoceptors by NA also reduces a resting potassium conductance, resulting in a pronounced slow depolarization. In this manner, NA and 5-HT may contribute to the switch from rhythmic burst firing to the transfer mode of action potential generation in the thalamus during increases in arousal and upon awakening from sleep.

INTRODUCTION

The cerebral cortex receives nearly all sensory information through the thalamus (Jones, 1985). The reliability and faithfulness with which synaptic inputs from the periphery are transmitted to the cortex by thalamic relay neurones varies with the state of the animal (Livingstone & Hubel, 1981; Steriade & Llinás, 1988). During slow wave sleep, or drowsiness, thalamic neurones discharge in a pattern of rhythmic bursts and are relatively unresponsive to stimulation of sensory receptive fields. During periods of alertness, thalamic neurones are either silent or spontaneously generating single action potentials and their excitability is greatly increased. Indeed, the degree to which thalamic relay neurones respond to peripheral inputs is directly related to the arousal state of the animal (Coenen & Vendrik, 1972; Livingstone & Hubel, 1981; Steriade & Llinás, 1988).

The switch from rhythmic burst firing and unresponsiveness to receptive field stimulation to tonic firing and greatly increased excitability arises from extra-thalamic inputs, of which the widespread projections from the locus coeruleus and median raphe nuclei are thought to contribute (Jouvet, 1972; Steriade & Deschênes, 1984; de Lima & Singer, 1987; Steriade & Llinás, 1988). These two nuclei are the source of noradrenergic and serotonergic innervation of the thalamus, respectively (Lindvall, Björklund, Nobin & Stenevi, 1974; Morrison & Foote, 1986; de Lima & Singer, 1987). In addition, the specific relay nuclei of the thalamus contain high densities of α_1-adrenergic receptors, and moderate densities of β-adrenergic and

serotonergic receptors (Jones, Gauger & Davis, 1985; Pazos, Cortes & Palacios, 1985; Pazos & Palacios, 1985).

In other neuronal systems, shifting of firing patterns from one stable state to another is achieved in part through the neuromodulation of distinct ionic currents localized in specific neuronal cell types (Llinás, 1988). Of particular relevance here, the hyperpolarization-activated cation current, I_f, in the heart has been proposed to contribute critically to the determination of heart rate and is modulated by both noradrenaline (NA) and acetylcholine (DiFrancesco, 1985; DiFrancesco & Tromba, 1988*a*, *b*; DiFrancesco, Ducouret & Robinson, 1989). Recently we have reported that application of NA or serotonin (5-HT) on thalamocortical relay neurones results in an increase in membrane conductance which may result from an enhancement of the hyperpolarization-activated cation current, I_h (Pape & McCormick, 1989). Here we investigate further the electrophysiological characteristics of this modulatory action of NA and 5-HT, evaluate the possible involvement of cyclic AMP as an intermediary in this response, and examine functional consequences of modulating I_h on neuronal responsiveness with particular reference to different functional states of the thalamus.

METHODS

Methods for preparing and maintaining thalamic slices for intracellular recordings and for application of neuroactive substances were the same as described in the accompanying paper (McCormick & Pape, 1990). All substances were obtained from Sigma except for prazosin (Pfizer), methysergide maleate (Sandoz), and ±8-hydroxy-dipropylaminotetralin (8-OHDPAT, Research Biochemicals Incorporated). Drugs were dissolved in bathing medium immediately before use; baclofen was diluted from a 1 mM stock solution, and forskolin and 1,9-dideoxy-forskolin were dissolved in ethanol as a stock solution (1 mM) and diluted to final concentrations just before use. L-Ascorbic acid was added to adrenergic agonists in equimolar concentration to prevent rapid oxidation (Hughes & Smith, 1978). Responses elicited by medium containing adrenergic agonists and ascorbic acid were identical to those elicited by adrenergic agonists alone. To isolate effects of noradrenaline through β-receptors, prazosin (2 μM) and yohimbine (1 μM) were routinely added to the bathing medium. Responses to serotonin or the β-receptor-specific agonist isoprenaline were identical in either normal or prazosin–yohimbine-containing medium. Applications of 5–15 pl of agonist to the surface of the slice within approximately 50 μm of the entry point of the recording electrode were sufficient to elicit large responses.

RESULTS

The results presented here were obtained from a similar sample of thalamic neurones as in the accompanying paper ($n = 139$; McCormick & Pape, 1990). In the absence of pharmacological antagonists, local application of NA (500 μM in micropipette) to the surface of the slice while recording from a presumed thalamocortical relay neurone at normal resting potential (−60 to −70 mV) resulted in a slow depolarization (Fig. 1*A*). Compensation for this slow depolarization with the intracellular injection of current revealed that it was associated with a decrease in apparent input conductance (Fig. 1*A* and *B*). We have shown previously that this response is mediated by α_1-adrenoceptors and represents a decrease in potassium conductance (McCormick & Prince, 1988). Similarly, we found here that local application of the α_1-specific antagonist prazosin (10 μM; $n = 6$) completely blocked

the slow depolarizing response to NA (cf. Fig. 1*A* and *C*). After block of α_1-receptors, application of NA to neurones depolarized to near firing threshold (−59 to −53 mV) resulted in a small increase in apparent input conductance (Fig. 1*C*). In contrast, application of NA with the neurone at resting membrane potential (−65 mV)

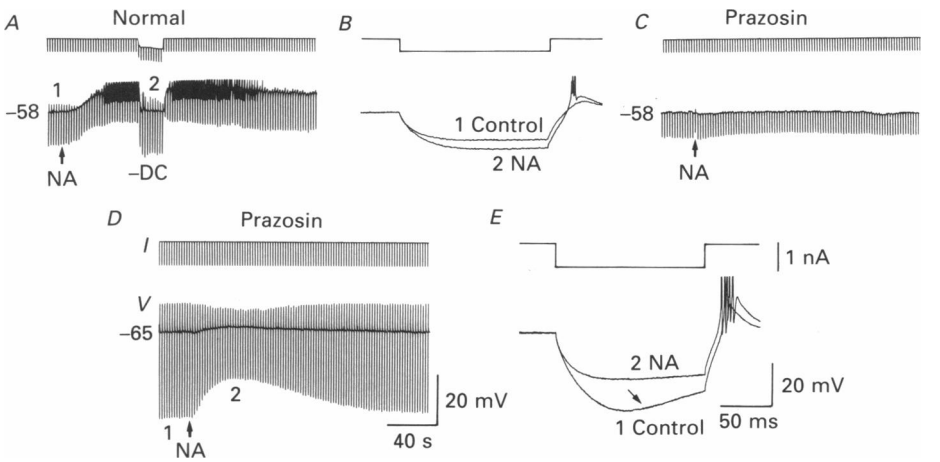

Fig. 1. Responses of LGND relay cells to noradrenaline. *A*, local application of noradrenaline (NA; 0·5 mM in pipette) after depolarization of the neurone to −58 mV with intracellular injection of current results in a slow depolarization that reaches neuronal firing threshold (amplitude of spikes are truncated). Compensation of the slow depolarization with the intracellular injection of hyperpolarizing current (−DC) reveals a decreased input conductance, which is due to a decrease in membrane potassium conductance (McCormick & Prince, 1988). The electrotonic responses induced by the constant-current pulse before (1) and after (2) NA are expanded for comparison in *B*. *C*, local application of the α_1-specific antagonist prazosin (10 μM) blocks the NA-induced slow depolarization. Application of NA now reveals a small increase in membrane conductance. *D*, application of NA with the cell at normal resting potential (−65 mV, as indicated), in the presence of prazosin, results in a small depolarization and a large increase in membrane conductance. Comparison of responses before (1) and during (2) the increase in membrane conductance are shown in *E*. Upper trace is current; lower trace is membrane potential in this and subsequent figures. Scale bars in *D* are for *A*, *C* and *D*; scale bars in *E* are for *B* and *E*.

resulted in a small (1–3 mV) depolarization and a substantial increase in apparent membrane conductance (Fig. 1*D*). Examination of the response to a hyperpolarizing current pulse before and during this adrenergic effect revealed a striking increase in the apparent input conductance (Fig. 1*E*).

This action is not restricted to NA, for application of 5-HT (300 μM; $n = 21$) had a similar or identical effect (Fig. 2*C*), although the response to 5-HT was usually smaller in amplitude and longer in duration. In addition, this response to NA and 5-HT is robust in that it was found in $> 90\%$ ($n = 132/139$) of presumed relay neurones tested in the cat and guinea-pig dorsal lateral and medial geniculate nuclei.

The pre- or postsynaptic location of receptors mediating this effect of NA and 5-HT was determined by blocking synaptic transmission with either local applications of the Na^+ channel poison, tetrodotoxin (TTX; 10 μM), or by reducing extracellular

Ca^{2+} concentration and adding Mn^{2+} to the bathing medium. Local application of TTX completely blocked synaptic potentials elicited by local electrical stimulation (Fig. 2*B*), but did not block the response elicited by stimulation of β-adrenergic receptors (Fig. 2*A*; $n = 4$). Similarly, the increase in apparent input conductance

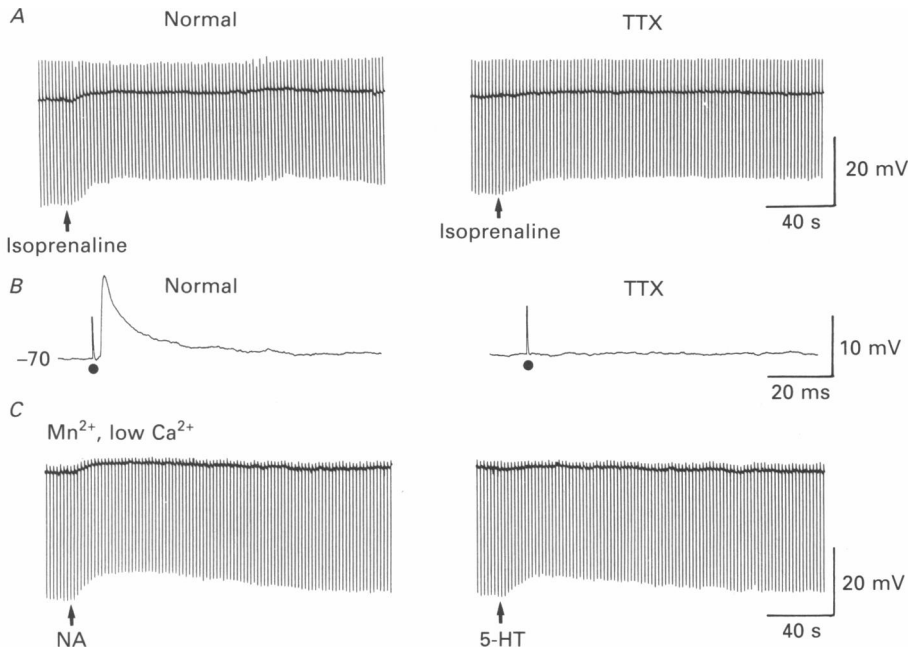

Fig. 2. Serotonergic- and β-noradrenergic-induced increases in input conductance are direct postsynaptic responses. Local application of the β-specific agonist isoprenaline (50 μM) elicits a typical increase in apparent membrane conductance at resting potential (*A*, left). Local application of TTX (10 μM) blocks the depolarizing postsynaptic potential elicited by local electrical stimulation of synaptic inputs (*B*) but not the response to isoprenaline (*A*). *C*, block of synaptic transmission (not shown) by introduction of manganese (Mn^{2+}, 4 mM) and reduction of calcium (Ca^{2+}, 0·5 mM) in the bathing medium does not block the response to noradrenaline (NA, left) or serotonin (5-HT, right). Prazosin (2 μM) and yohimbine (1 μM) included in the bathing medium to block α-adrenergic responses.

elicited by NA and 5-HT persisted after block of synaptic transmission by reducing $[Ca^{2+}]_o$ to 0·5 mM and adding 4–8 mM-Mn^{2+} to the bathing medium (Fig. 2*C*; $n = 4$). These results indicate that the small depolarization and increase in membrane conductance mediated by NA and 5-HT result from the direct stimulation of post-synaptic receptors.

The pharmacological identification of receptors mediating this response of NA and 5-HT was investigated through the use of specific agonists and antagonists. Application of the 5-HT_1 and 5-HT_2 antagonist, methysergide (1–5 μM in the bath, $n = 4$; 10–100 μM in micropipette, $n = 4$), resulted in a complete block of the response to 5-HT (Fig. 3*A*). By contrast, the 5-HT_{1A} agonist, ipsapirone (0·2 mM), the partial agonist 8-OHDPAT (0·1–1 mM; $n = 4$), and the 5-HT_2 antagonist,

ritanserin (5 μM in bath; $n = 4$), all had no effect on the response of dorsal lateral geniculate nucleus (LGND) neurones to 5-HT. During blocked responses to 5-HT by methysergide, NA still evoked the increase in input conductance (Fig. 3*B*). This response to NA was reversibly blocked by application of the β-adrenoceptor

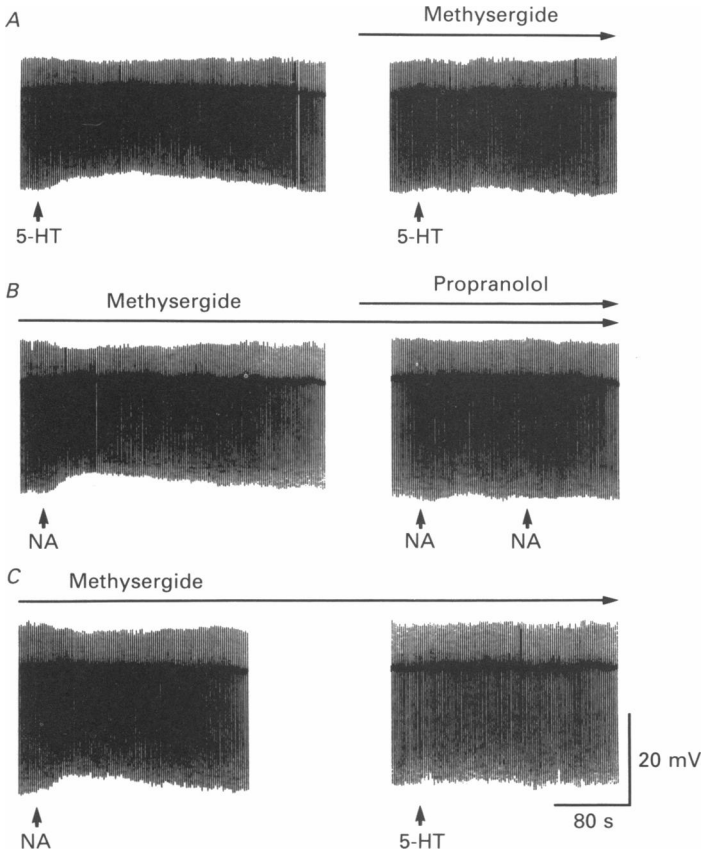

Fig. 3. Serotonergic and β-adrenergic responses are mediated through pharmacologically distinct receptors. *A*, local application of a high concentration of the serotonergic antagonist methysergide (100 μM in micropipette) blocks the response to local application of serotonin (5-HT, 0·3 mM in micropipette). *B*, during blocked serotonergic responses, local application of noradrenaline (NA) elicits a typical response that is blocked by local application of the β-specific antagonist propranolol (100 μM in micropipette). *C*, recovery of responses to noradrenaline while responses to serotonin remain blocked.

antagonists, propranolol (100 μM in micropipette; $n = 2$; Fig. 3*B* and *C*) or atenolol (15 μM in bath; $n = 2$). This response to NA was not mediated by α_1-receptors or α_2-receptors, since prazosin (2 μM) and yohimbine (1 μM) were routinely added to the bathing medium. In addition, the slow increase in input conductance was mimicked by local application of the β-receptor-specific agonist, isoprenaline (50–250 μM; $n = 54$; see Figs 2*A* and 8*A*).

These data indicate that the NA-induced increase in apparent input conductance in the hyperpolarizing range occurs through β-adrenoceptors. Therefore, in all of the

following experiments, the α_1 antagonist prazosin (2 μM) and the α_2 antagonist yohimbine (2 μM) were routinely contained in the bathing medium in order to isolate the actions of NA through β-adrenoceptors. Although our results do not positively identify which of the many serotonergic receptor subtypes mediate this response to

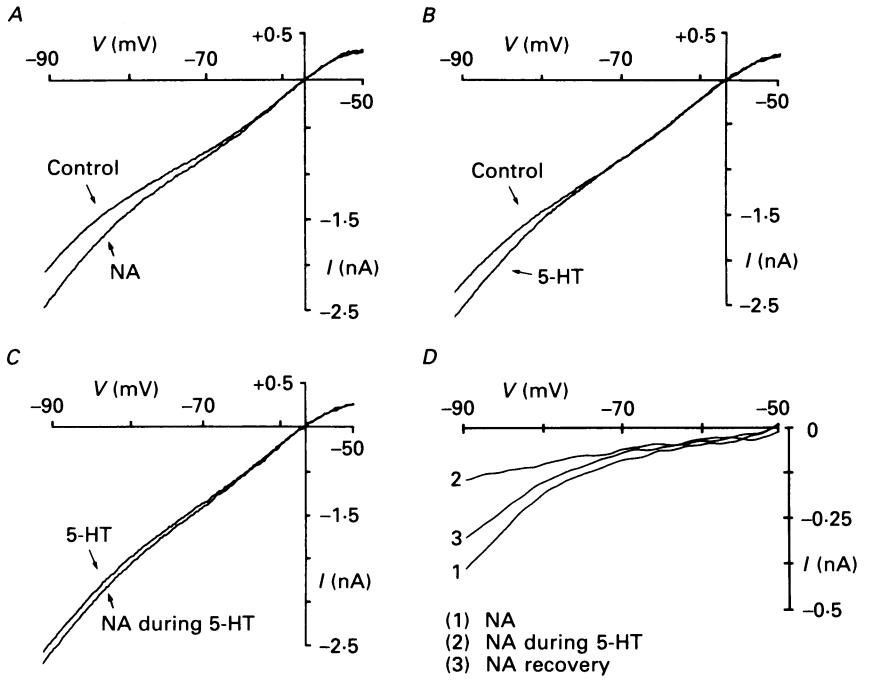

Fig. 4. Effects of NA and 5-HT on *I–V* relationships of LGND neurones obtained under voltage clamp. Application of either NA (0·5 mM; *A*) or 5-HT (0·3 mM; *B*) enhanced inward rectification at membrane potentials negative to approximately −60 mV. This effect may represent a common postsynaptic response to these two agonists since maximal stimulation of 5-HT receptors strongly reduced the effects of NA on the *I–V* relationship (*C*). Subtracting the *I–V* relationships before and after application of NA resulted in an *I–V* plot of the NA-induced current (*D*). These difference curves (*D*) illustrate the NA-induced inward current (1; constructed from the difference of *I–V* curves before and during action of NA, cf. *A*), its reduction during action of 5-HT (2; difference curve from *I–V* curves in *C*), and its recovery after wash-out of 5-HT (3). *I–V* relationships were obtained under voltage clamp conditions by continuously moving the membrane potential from a holding potential of −50 mV to a final potential of −100 mV and measuring the amount of current required to do so.

5-HT, they suggest that it may be either a subtype of 5-HT_1 receptor or an as of yet unidentified 5-HT receptor (Peroutka, 1988).

Voltage dependence of NA and 5-HT actions

The dependence of the NA- and 5-HT-induced increase in membrane conductance on the membrane potential of the cell (e.g. Fig. 1) indicated that these two putative neurotransmitters may be altering a voltage-dependent current. To investigate this

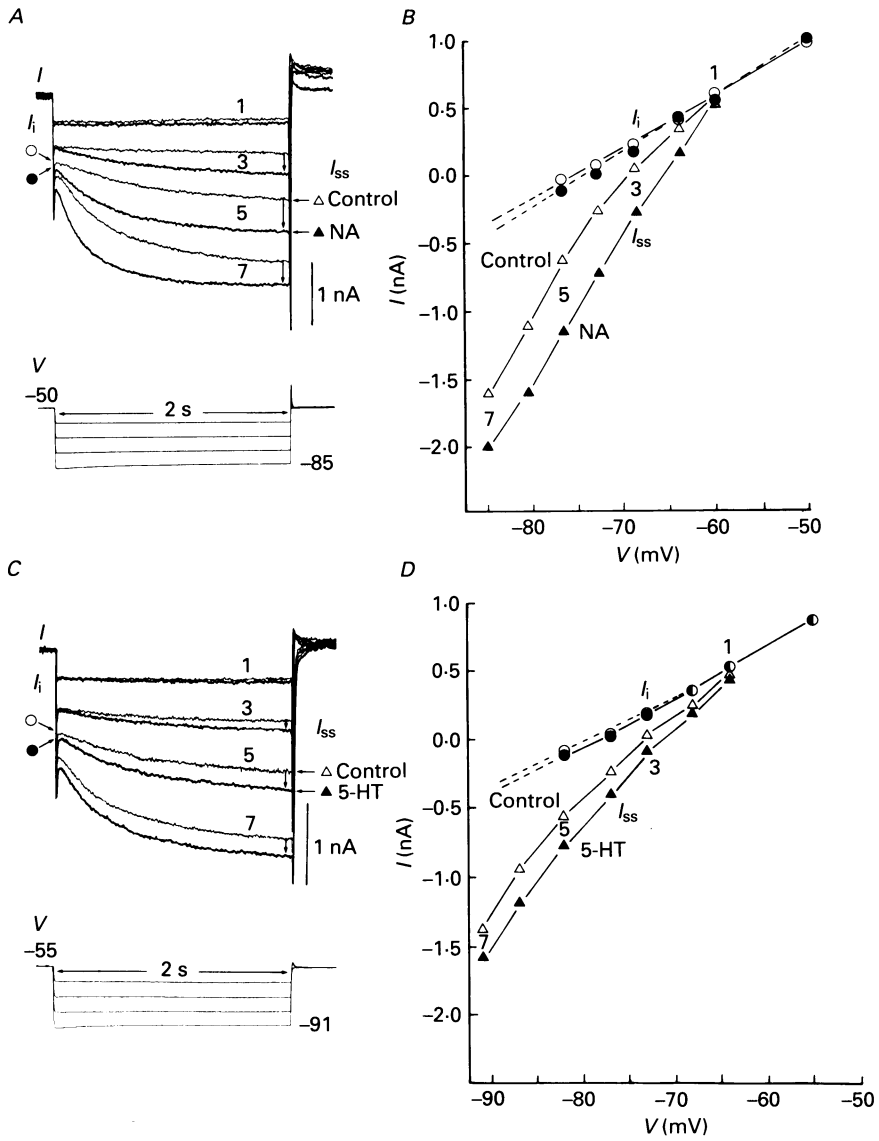

Fig. 5. Noradrenaline and serotonin increase I_h. *A*, family of currents (upper traces) evoked in a LGND relay cell by incremental voltage steps from a holding potential of −50 mV to −85 mV (as indicated near lower traces). The smaller current in each pair (see arrow) was recorded under control conditions; the larger current was elicited by the same voltage step during action of NA (0·5 mM). NA increases the time- and voltage-dependent inward current (I_{ss}) with little effect on instantaneous current (I_i). *B*, *I–V* relationship obtained from experiment in *A*, including the complete family of voltage steps. I_i plotted as circles and I_{ss} plotted as triangles; currents obtained under control conditions represented as open symbols, during NA as filled symbols. Numbers near symbols depict examples shown in *A*. *C*, same experimental protocol as in *A*. Application of 5-HT (0·3 mM) increases I_{ss} with a small increase in I_i. *D*, *I–V* relationship obtained from experiment in *C*, including complete family of voltage steps. Symbols for I_i and I_{ss} as in

possibility, we obtained current *versus* voltage relations under voltage clamp conditions before and during the influence of NA and 5-HT on thalamic relay neurones. Both neuroactive substances were found to cause a voltage-dependent inward shift of the *I–V* relation at membrane potentials negative to approximately −60 to −70 mV (Fig. 4*A* and *B*). Subtracting control *I–V* relationships from those obtained during the action of NA or 5-HT revealed a striking voltage dependence of this effect, with the response becoming progressively larger as the cell was steadily hyperpolarized (e.g. Fig. 4*D*1).

The marked similarity between the response to NA and 5-HT indicated that they may be converging onto the same ionic current. This hypothesis is supported by the finding that a maximal application of 5-HT under voltage clamp conditions greatly reduced or abolished responses to NA (Fig. 4*C* and *D*; $n = 4$).

Among the plethora of voltage-gated conductances inherent in thalamocortical relay neurones (Jahnsen & Llinás, 1984*a*, *b*), those that are active in a voltage region negative to the normal resting potential are the most likely candidates to be modulated by NA and 5-HT, as is indicated by the unique voltage dependence of the response. This possibility was investigated by examining the effects of NA and 5-HT on the currents activated by hyperpolarizing voltage steps to between −60 and −110 mV, from holding potentials slightly below firing threshold (i.e. −55 mV). Stepping to membrane potentials negative to approximately −60 mV elicited a slow inward current, I_h, as described in the accompanying paper (McCormick & Pape, 1990). Application of NA (Fig. 5*A* and *B*; $n = 20$) or 5-HT (Fig. 5*C* and *D*; $n = 5$) resulted in a marked enhancement of I_h, with little, if any, change in instantaneous conductance at the beginning of the hyperpolarizing pulse. In general, effects of 5-HT on I_h were smaller in amplitude when compared with those exerted by NA.

Examination of the current traces before and in the presence of NA or 5-HT revealed that the time constant (τ) with which I_h activated appeared to be decreased (Fig. 5*A* and *C*). Single-exponential fits of I_h before and after application of NA revealed that this agonist induced a substantial decrease in the time constant with which I_h activated (Fig. 6; $n = 5$). This decrease varied with voltage, such that in the cell in Fig. 6 at −77 mV, τ decreased from approximately 2 s to 900 ms, while at −104 mV, τ decreased from 260 ms to 180 ms.

Effect of extracellular Cs^+ and Ba^{2+} on the response to β-adrenoceptor stimulation

The hyperpolarization-activated cation current, I_h, is blocked by locally or bath-applied Cs^+ but not Ba^{2+} (McCormick & Pape, 1990). If the increased input conductance following stimulation of β-adrenoceptors is based upon an increase of I_h, locally applied Cs^+ should block this effect while Ba^{2+} should not. Indeed, local application of Cs^+ (2–10 mM; $n = 12$) to relay cells resulted in a 1–10 mV hyperpolarization from normal resting potential, a decrease in apparent input conductance (Fig. 7*A*), a reduction or block of I_h (Fig. 7*C*), and a complete block of

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B; open during control, filled during 5-HT. I_i during larger hyperpolarizations was extrapolated (dashed line) from instantaneous *I–V* relationship at smaller hyperpolarizations, where direct measurement of the current was possible. Tail currents contaminated by transient Ca^{2+} and K^+ currents.

responses to isoprenaline (Fig. 7*A*) as well as to NA and 5-HT (not shown). The specificity of this effect is illustrated by the finding that local applications of Cs^+ which blocked I_h had no effect on the response to local application of γ-aminobutyric acid (GABA; 1 mM; Fig. 7*B*). In contrast, local (1 mM) or bath (500 μM) application

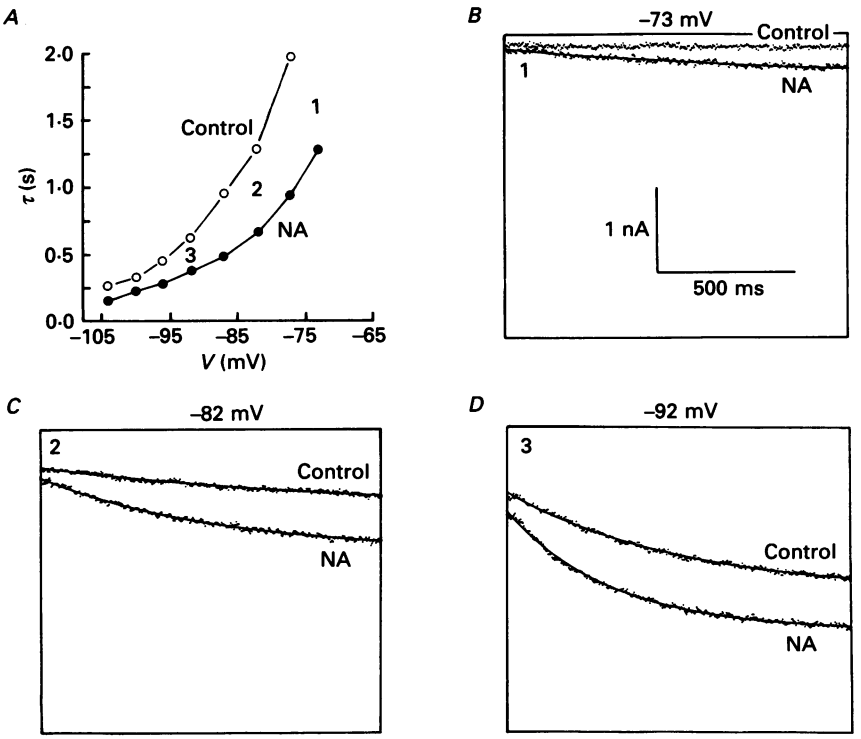

Fig. 6. Noradrenaline enhances the activation rate of I_h. *A*, plot of time constant (τ) of I_h activation *versus* membrane potential under control condition (○) and during action of NA (●) as determined through single-exponential fits (*B–D*). Time constants are strongly voltage dependent and are decreased by NA over the entire voltage range tested. *B–D*, examples of inward currents elicited by stepping from −50 mV to −73 mV (*A*), −82 mV (*C*) and −92 mV (*D*) under control conditions and with NA, as indicated. The currents are plotted as dots while the single-exponential fits are plotted as lines.

of Ba^{2+} ($n = 4$) did not block the response to NA even though increases in potassium conductance induced by local application of the $GABA_B$ agonist baclofen (0·1 mM; Fig. 8) or by the muscarinic agonist methacholine (see Fig. 7 in McCormick & Pape, 1990) were abolished. These results indicate that the increase in apparent input conductance in the hyperpolarizing range after stimulation of β-adrenergic receptors is due to alterations in the properties of I_h.

Effects of NA on G_h activation curve

In the heart (DiFrancesco, 1985) and in sympathetic ganglion cells (Tokimasa & Akasu, 1990), stimulating β-adrenergic receptors and/or increasing the intracellular concentration of cyclic AMP both induce a shift towards more positive values in the

activation range of a hyperpolarization-activated cation current. Similarly, we found that application of isoprenaline resulted in a 4–6 mV positive shift of the activation curve of the conductance underlying I_h (G_h) on the voltage axis (Fig. 9; $n = 5$). Due to the strong voltage dependence of the time course of I_h activation, this positive

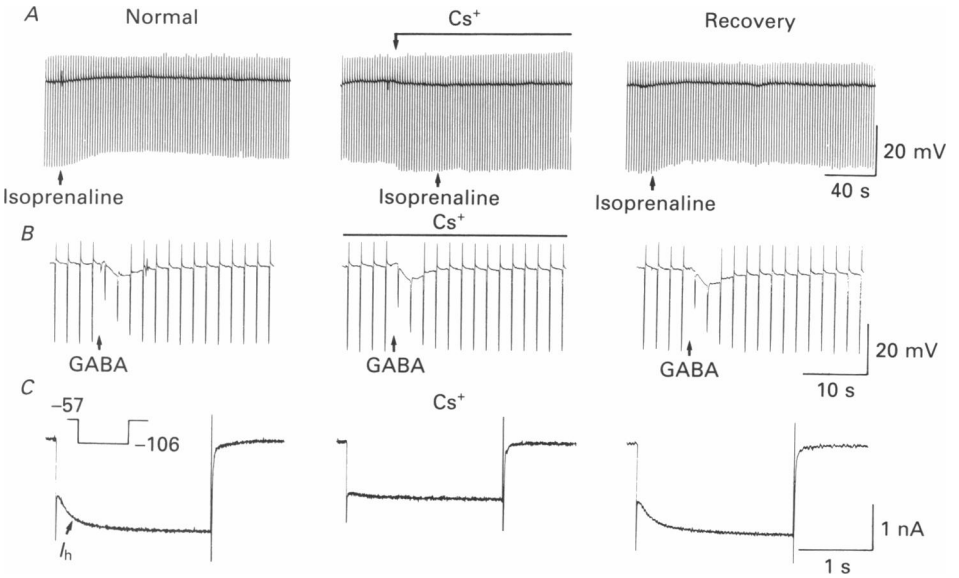

Fig. 7. Application of extracellular caesium (Cs^+) blocks β-adrenergic responses in parallel with blockade of I_h. *A*, typical response elicited by isoprenaline (250 μM, local application) at resting potential is reversibly blocked by local application of Cs^+ (10 mM in micropipette). Note small hyperpolarization and decreased input conductance during Cs^+. *B*, hyperpolarizing response to GABA (1 mM, local application) is not affected by Cs^+. *C*, slow inward current (I_h, arrow) elicited by stepping the membrane potential from -57 mV to -106 mV (inset) is reversibly blocked by Cs^+. Note unchanged instantaneous current at the beginning of the pulse. All recordings were made in the same LGND neurone.

shift along the voltage axis of the activation curve might account for the substantial increase in amplitude of I_h as well as the decreased time constant of activation that was observed for a given voltage before and during application of NA (e.g. Figs 5 and 6). Another important aspect of this shift of the activation curve of G_h is the value of this conductance which is active at resting membrane potentials. Because of the steepness of the activation curve, a positive shift of only a few millivolts will result in a substantial increase in the resting membrane conductance of the neurone (Fig. 9), which may have important consequences for the responsiveness of thalamocortical relay neurones (see below).

Increase by NA of amplitude of I_h active at resting membrane potential

A somewhat unexpected feature of the response to activation of β-adrenergic and serotonergic receptors is the finding that in current clamp recordings the response of the neurone to the entire hyperpolarizing pulse becomes smaller, even to an extent

in which the sag back towards resting membrane potential is greatly decreased (see Fig. 1*E*, arrow). One possible explanation of this finding is that the apparent input conductance of the cell is increased due to an increase in the amplitude of G_h that is tonically activated at rest. This hypothesis is supported by five different findings.

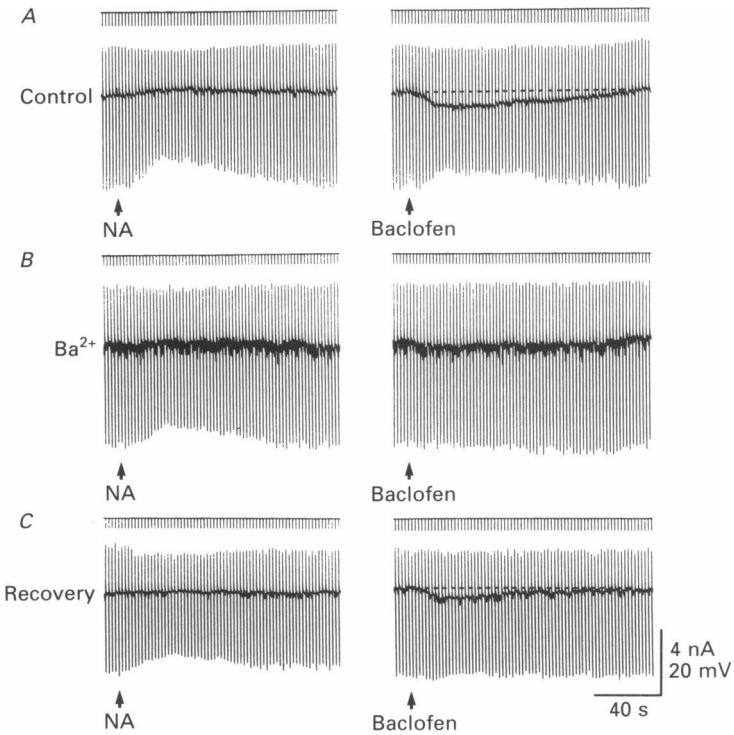

Fig. 8. β-Adrenergic response is not antagonized by extracellular barium. *A*, local application of NA (0·5 mM) elicits typical slow increase in membrane conductance while application of the $GABA_B$ agonist baclofen (100 μM) elicits a hyperpolarization, due to an increase in potassium conductance (Hirsch & Burnod, 1987; Crunelli *et al.* 1988). *B*, introducing barium (0·5 mM) in the superfusate increases synaptic activity (cf. increased background noise), and blocks $GABA_B$-mediated, but not β-adrenergic, responses. *C*, recovery after washing barium from the bath. All data are from the same LGND neurone.

First, the β-adrenergic shifts in the activation curve of G_h (Fig. 9) are associated with substantial increases in the degree to which G_h is active at resting membrane potentials (e.g. -65 mV). Secondly, local application of Cs^+ (10 mM in micropipette) to thalamocortical relay neurones at rest consistently result in hyperpolarization and a decrease in membrane conductance (Fig. 10*A*). Depolarization of the neurone to -55 mV resulted in an abolition of this effect (Fig. 10*B*), probably due to a lack of a 'standing' G_h at this membrane potential (as indicated by activation curves). Thirdly, holding neurones near firing threshold (-50 to -55 mV) in voltage clamp mode and stepping to the resting potential (as indicated by zero-current reading at the end of the step) resulted in the activation of a slow inward current. Local application of Cs^+ completely blocked this current, characterizing the current as I_h

(Fig. 10*C*). Fourthly, stepping from firing threshold to resting membrane potential in voltage clamp elicited I_h, the amplitude and rate of activation of which were greatly increased by NA (Fig. 10*F*). Finally, application of NA to neurones depolarized to near firing threshold resulted in a much smaller increase in apparent

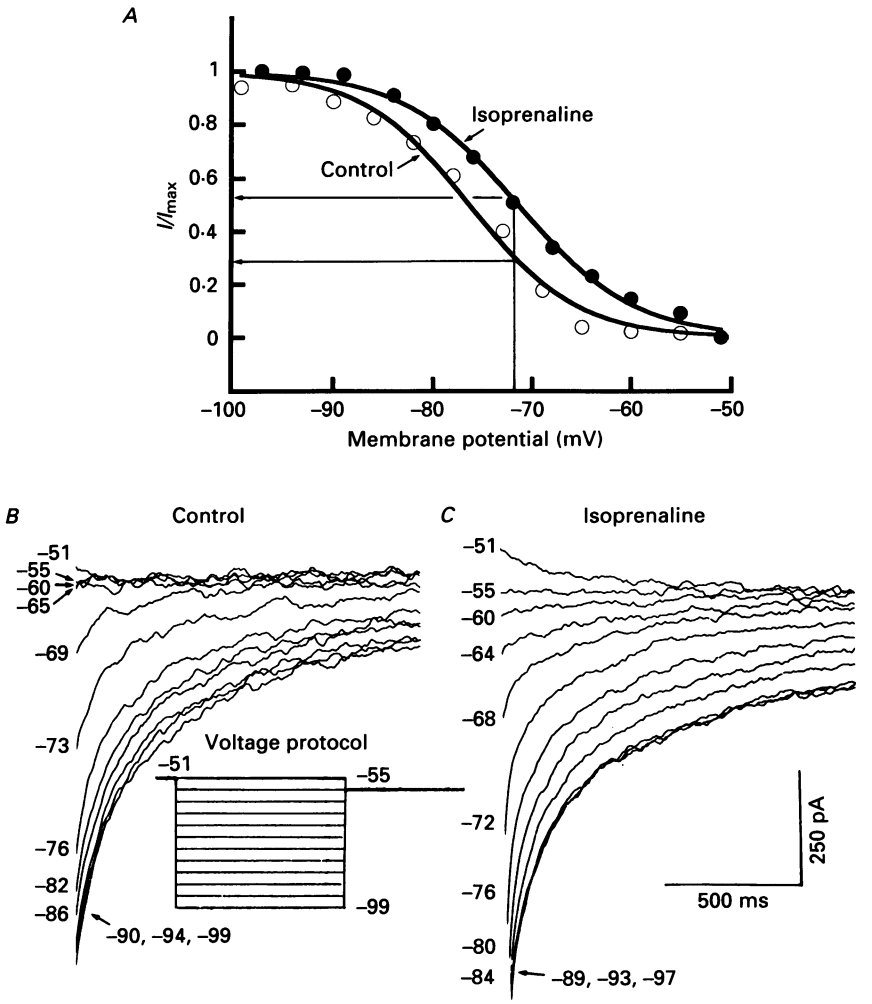

Fig. 9. Effects of NA on I_h activation curve. *A*, activation of β-adrenergic receptors with isoprenaline (0·3 mM in micropipette) results in a shift of the activation curve for G_h by approximately 5 mV with no apparent change in slope. Arrows indicate the proportion of G_h which was active at resting membrane potential (−72 mV) in this neurone before and after application of isoprenaline. *B* and *C*, tail currents during de-activation of I_h at −55 mV used to calculate data illustrated in *A*. Voltage step protocol is illustrated in *B*.

input conductance than that seen at resting membrane potentials (Fig. 10*D* and *E*), presumably due to the strong voltage dependence of G_h. Unlike the complete abolition of Cs^+ effects, NA still induced a small increase in input conductance at depolarized potentials (compare Fig. 10*B* and *E*). A possible explanation of the

persistence of this effect is the NA-induced positive shift of the G_h activation curve along the voltage axis that might allow recruitment of this conductance at more positive potentials (Fig. 9; see Discussion). In summary, these results indicate that activation of β-adrenoceptors leads to a substantial increase in apparent membrane

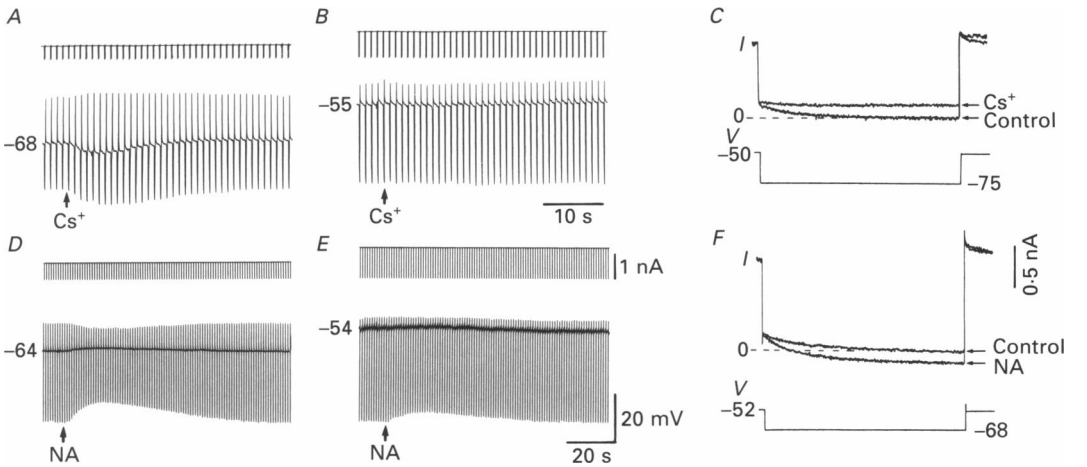

Fig. 10. Comparison of the actions of Cs^+ and NA at different membrane potentials under current clamp (*A*, *B*, *D* and *E*) and voltage clamp (*C* and *F*) conditions. *A* and *B*, application of Cs^+ (10 mM, local) induces a membrane hyperpolarization and decreased input conductance when applied at normal resting potential (−68 mV; *A*), but has no effect when applied with the cell near firing threshold (−55 mV; *B*), even though the amplitudes of the hyperpolarizing pulses were increased to result in the same peak electrotonic response. *C*, hyperpolarizing voltage steps from −50 mV to resting membrane potential (−75 mV; as indicated by zero-current level) reveal that Cs^+ reduces the slow inward current (I_h) with no effect on instantaneous current. *D* and *E*, application of NA (0·5 mM, local) induces a typical β-adrenergic response when applied at normal resting potential (−64 mV; *D*), that is strongly reduced when applied at a depolarized membrane potential (−54 mV; *E*). *F*, hyperpolarizing voltage steps from −52 mV to resting membrane potential (−68 mV) demonstrate the NA-induced increase of I_h. Data obtained from four different LGND neurones (*A*, *B*; *C*; *D*, *E*; and *F*).

conductance in large part by increasing the amount of G_h active at resting membrane potential.

Involvement of cyclic AMP in NA- and 5-HT-induced increases of I_h

In many regions of the central and peripheral nervous system stimulation of β-adrenergic receptors leads to activation of adenylyl cyclase and consequently an increase in the intracellular concentration of cyclic AMP (reviewed in Kupferman, 1980). In the heart, increases in cyclic AMP appear to mediate the β-adrenergic enhancement of the hyperpolarization-activated cation current, I_f (Hagiwara & Irisawa, 1989). Here we investigated the possible involvement of cyclic AMP in the enhancement of I_h by NA and 5-HT.

Local application of either the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX, 250 μM; $n = 4$), the membrane-permeable cyclic AMP analogue,

8-bromo-cyclic AMP (500 μM; $n = 6$), or the adenylyl cyclase stimulant, forskolin (25 μM; $n = 5$), were all found to result in a substantial increase in I_h with little change in the instantaneous currents expressed at the beginning of the voltage steps (Fig. 11). Recent investigations have indicated that forskolin can modulate specific

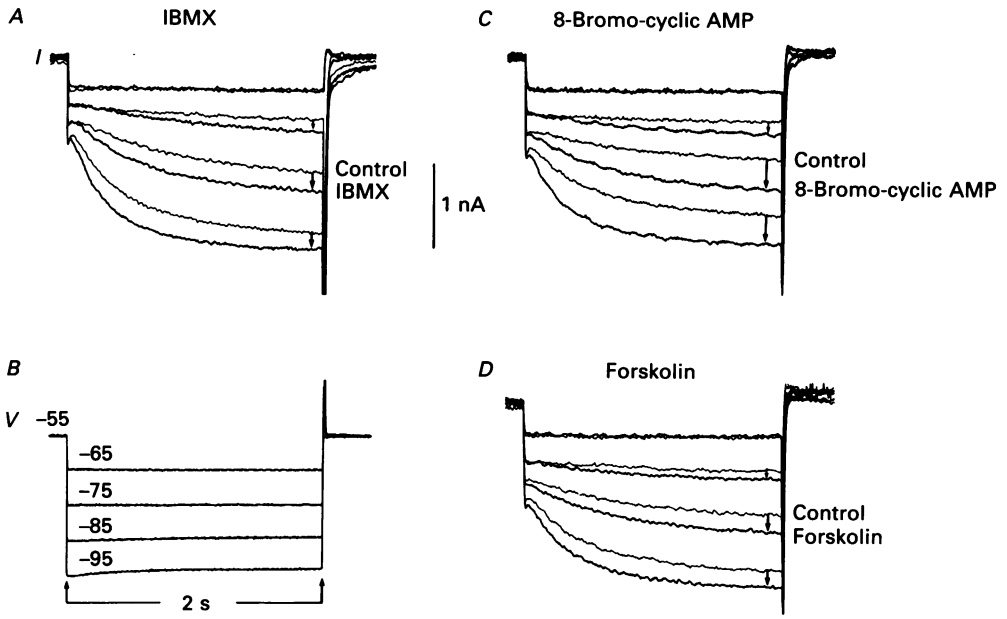

Fig. 11. Increases in intracellular cyclic AMP enhance I_h. Local application of the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX, 300 μM in pipette; *A*), the membrane-permeable cyclic AMP analogue 8-bromo-cyclic AMP (500 μM; *C*), or the adenylyl cyclase stimulant forskolin (25 μM; *D*) all result in a marked enhancement of I_h with no significant change in instantaneous current. Voltage clamp data from the same LGND cell; voltage steps are shown in *B* (numbers indicate holding and step potential in millivolts); respective currents are shown in *A*, *C* and *D*. Smaller current in each pair (see arrow) recorded under control conditions. Recovery was obtained after each drug.

ionic currents through a mechanism which does not entail activation of cyclic AMP (Hoshi, Garber & Aldrich, 1988; Wagoner & Pallota, 1988). To test for this possibility, we applied 1,9-dideoxy-forskolin, a forskolin analogue which only weakly stimulates adenylyl cyclase (Seamon & Daly, 1986). Applications of 1,9-dideoxy-forskolin (50 μM; $n = 4$) resulted in either no, or just-detectable, increases in I_h in cells in which subsequent applications of forskolin at half the concentration exhibited the full effect (not shown).

Functional consequences of neurotransmitter enhancement of I_h

Rhythmic burst firing in the thalamocortical relay neurones *in vivo* occurs in two basic forms: (1) spindle waves, which are characterized by the arrival of IPSPs at a rate of 8–12 Hz (Steriade & Deschênes, 1984) and (2) rhythmic 1–2 Hz burst firing (Lamarre, Fillion & Cordeau, 1971; Steriade, Deschênes, Domich & Mulle, 1985). Both of these types of oscillation occur within the voltage range of I_h, implying that

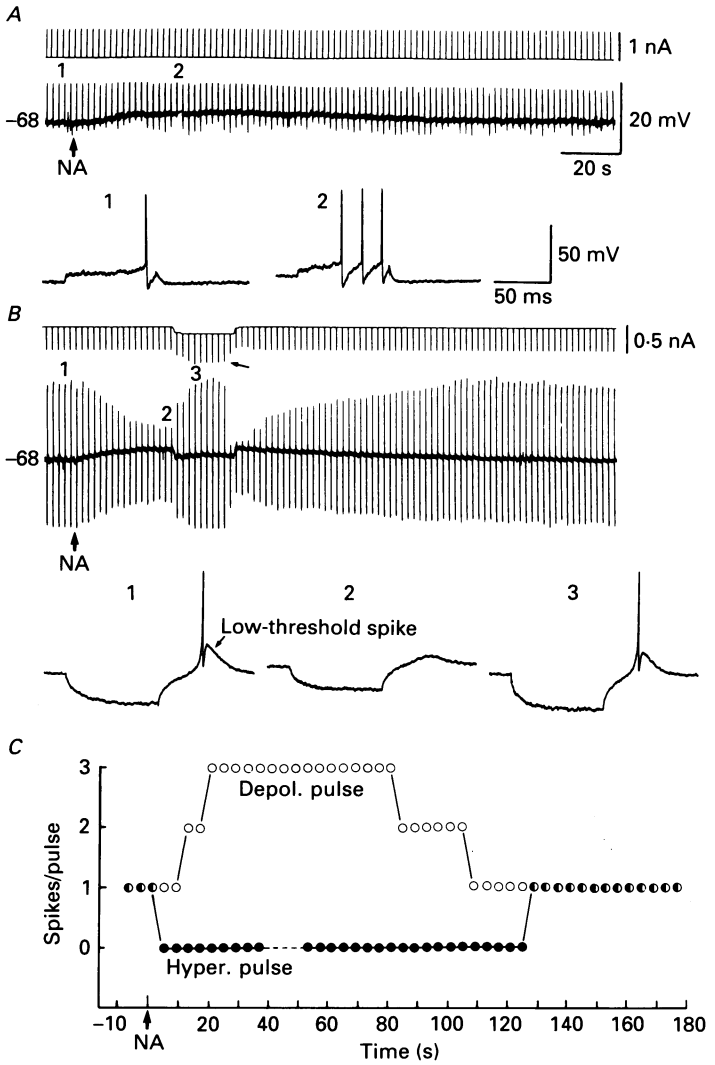

Fig. 12. Consequences of activation of β-adrenergic receptors on neuronal responses to depolarizing and hyperpolarizing current pulses. *A*, injection of depolarizing current pulses (1 nA, 80 ms) into a cell at resting membrane potential (−68 mV). Each current pulse reached threshold for activation of a single spike as depicted at a faster time scale (*A*1). Application of NA induces a small depolarization and facilitation of spike firing (*A*2). *B*, application of NA results in a typical increase in apparent input conductance which reduces rebound Ca^{2+} spikes. Periodic injection of a hyperpolarizing current pulse (0·5 nA, 80 ms) is followed by a low-threshold Ca^{2+} spike which reaches threshold for the generation of one fast action potential (*B*1). Application of NA results in a membrane depolarization, reduced hyperpolarizing response to each current pulse, and decreased rebound low-threshold Ca^{2+} spike (*B*2). Compensation for the small depolarization with the intracellular injection of current and increasing the amplitude of the pulse to result in the same voltage deviation prior to NA restores the pre-NA firing pattern (*B*3). *C*, number of fast spikes evoked by each depolarizing current pulse in experiment *A* (○) and number of rebound spikes evoked by each hyperpolarizing current pulse in experiment *B* (●) are plotted against time. Application of NA (at zero time) blocks rebound spike

NA- and 5-HT-induced shifts in the activation curve for this current may have profound effects on this type of activity. This possibility was tested either by examining the effects of β-receptor stimulation on the response of non-oscillating thalamocortical relay neurones to the periodic (0·5–4 Hz) injection of 40–120 ms duration hyperpolarizing and depolarizing current pulses (Fig. 12) or by examining directly, in oscillating cells, the influence of 5-HT or NA on endogenous burst firing (Fig. 13).

Application of NA or isoprenaline to LGND neurones during the injection of current pulses which elicited one action potential each resulted in a small depolarization and an increase in the number of evoked spikes to two or more (Fig. 12; compare *A*1 and *A*2). This increase in the number of evoked action potentials appeared to result from the depolarization of the membrane, since hyperpolarization of the membrane back to resting values reinstated the response to one action potential (not shown).

Due to the voltage dependence and kinetics of the low-threshold Ca^{2+} current present in thalamocortical relay neurones (Coulter, Huguenard & Prince, 1989; Crunelli, Lightowler & Pollard, 1989; Hernández-Cruz & Pape, 1989), a slow calcium action potential can be elicited as a rebound at the offset of a hyperpolarizing current pulse (e.g. Fig. 12*B*1) and can activate one to six classical Na^+–K^+-mediated action potentials (Jahnsen & Llinás, 1984*a*, *b*). The voltage range of de-inactivation of this Ca^{2+} current is well within the range of activation of the cation current, I_h. Thus, modulation of I_h may influence the generation of rebound burst discharges in thalamic neurones. This was tested through injections, at the resting potential, of hyperpolarizing current pulses that removed enough inactivation of the low-threshold Ca^{2+} spike to reach threshold for generation of a single fast spike (Fig. 12*B*1). Application of NA under these conditions resulted in a small depolarization and increase in apparent input conductance. The associated decrease in hyperpolarizing response to the current pulse resulted in a marked decrease in the rebound Ca^{2+} spike which now failed to generate a fast action potential (Fig. 12*B*2). Repolarization of the membrane to the control value with intracellular injection of current (arrow) revealed that this effect resulted both from the depolarization of the membrane and from the increase in apparent input conductance (Fig. 12*B*). Increasing the amplitude of the hyperpolarizing current pulse to match the voltage deviation before application of NA reinstated the full rebound Ca^{2+} spike (Fig. 12*B*3). These results indicate that NA decreases rebound burst firing in part through depolarization of the membrane as well as through a limitation of neuronal responses to hyperpolarizing inputs.

The direct functional consequences on intrinsic thalamic oscillations of NA- and 5-HT-induced enhancement of I_h were investigated through extracellular and intracellular recordings. Extracellular recordings in the cat LGND revealed the presence of rhythmic bursts of action potentials which occurred on a very regular

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activity, and facilitates the number of spikes generated by each depolarizing stimulus. Period of compensation with current injection during experiment *B* not included (dashed line). Data are from the same LGND cell with prazosin (2 μM) and yohimbine (1 μM) included in the bathing medium.

basis of about 1–2 Hz in a subset of presumed thalamocortical relay neurones (Fig. 13*A*1), as has been reported previously (Haby, Leresche, Jassik-Gerschenfeld, Soltesz & Crunelli, 1988; McCormick & Prince, 1988; McCormick & Pape, 1990). Local application of small amounts of either isoprenaline ($n = 5$) or 5-HT ($n = 8$)

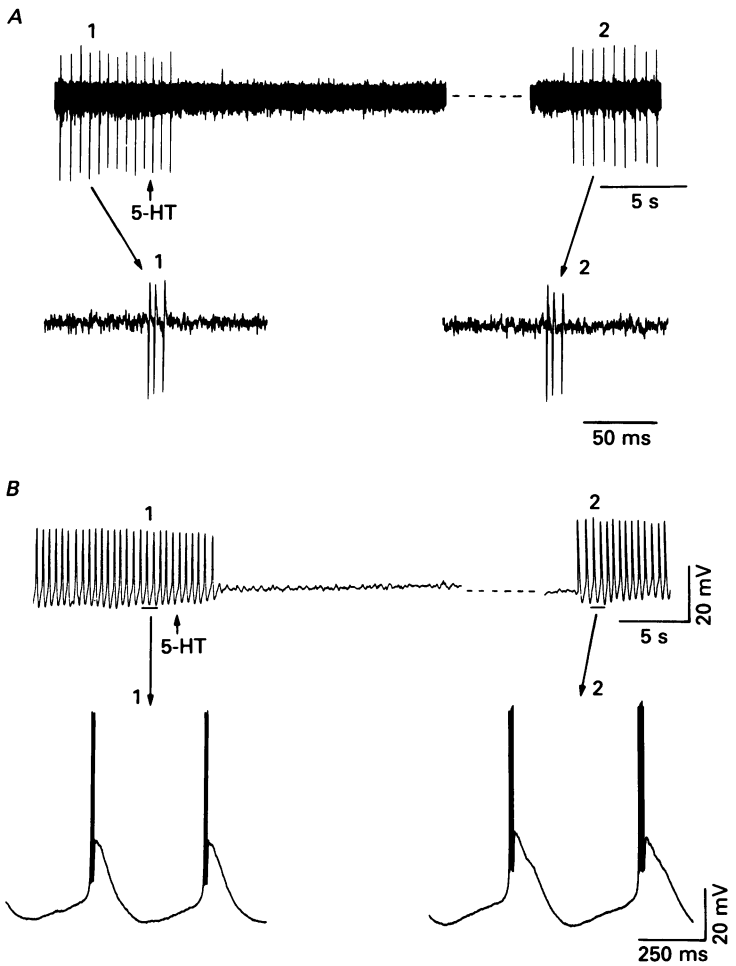

Fig. 13. Serotonin (5-HT) blocks rhythmic burst firing in thalamic neurones. *A*, application of 5-HT to a rhythmically bursting cat LGND neurone recorded extracellularly reversibly inhibits this activity. Each vertical line in the top trace in *A* represents between two and four action potentials, which are expanded in *A*1 and *A*2 for detail. *B*, application of 5-HT to rhythmically oscillating cat LGND neurone recorded intracellularly reversibly inhibits this activity. Segments *B*1 and *B*2 are expanded for detail.

resulted in a complete abolition of this rhythmic burst firing, subsequently resulting in a silent recording (Fig. 13*A*). After approximately 1–2 min, the rhythmic burst firing reappeared (Fig. 13*A*2).

Intracellularly, rhythmic burst firing is associated with the periodic and regular

occurrence of a low-threshold Ca^{2+} spike, which activates from zero to six Na^+- and K^+-mediated fast action potentials (Fig. 13*B*1). Each Ca^{2+} spike is triggered on the rising phase of a hyperpolarizing potential which represents in large part an increased activation of I_h (McCormick & Pape, 1990). Application of 5-HT or isoprenaline was associated with a dampening of this rhythmic activity (Fig. 13*B*) and a replacement of it with a small depolarization. As the depolarization lessened, rhythmic burst firing reappeared after about 1–2 min (Fig. 13*B*2).

DISCUSSION

The present results confirm and expand the finding that the hyperpolarization-activated cation current, I_h, is modulated by putative neurotransmitters in thalamocortical relay neurones (Pape & McCormick, 1989). Stimulation of both β-adrenergic receptors by NA and serotonergic receptors by 5-HT resulted in a marked enhancement of I_h. Findings which support this view are as follows: (1) the current *versus* voltage (*I–V*) relationship of the NA- or 5-HT-induced current is very similar to the *I–V* relationship obtained for I_h; (2) NA or 5-HT selectively increase the size and time course of activation of I_h with no consistent change in instantaneous current at a holding potential out of the range of I_h activation; (3) the activation curve of I_h is shifted by 4–6 mV on the voltage axis during stimulation of β-adrenoceptors; (4) low concentrations of extracellular Cs^+ selectively abolish I_h in parallel with blockade of responsiveness to NA or 5-HT, whereas Ba^{2+} does not affect I_h nor responses to NA or 5-HT.

Previous experiments have shown that stimulation of β-noradrenergic and some subtypes of serotonergic receptors can lead to activation of adenylyl cyclase in the central nervous system (Kupferman, 1980; Fayolle, Fillion, Barone, Oudar, Rousselle & Fillion, 1988). Similarly in thalamic neurones, the effects of NA and 5-HT may be mediated by an increase in the intracellular concentration of cyclic AMP since activation of adenylyl cyclase, reduction in phosphodiesterase activity (which would presumably result in an increase in intracellular cyclic AMP levels due to a decrease in its inactivation), and application of a membrane-permeable analogue of cyclic AMP all result in a marked enhancement of I_h. Involvement of cyclic AMP in enhancement of a hyperpolarization-activated cation current has previously been reported to occur in frog sympathetic ganglia (Tokimasa & Akasu, 1990), during stimulation of β-receptors in the heart (Hagiwara & Irisawa, 1989) and after activation of serotonin receptors on neurones in the prepositus hypoglossi nucleus of the brain stem (Bobker & Williams, 1989). These data indicate that the results presented here may have implications for the mechanisms of neurotransmitter function in wide regions of both the peripheral and central nervous system.

Consequences of monoaminergic modulation of I_h on depolarizing and hyperpolarizing neuronal responses

The positive shift in the I_h activation curve and increase in the rate of I_h activation resulting from β-adrenergic receptor stimulation suggest that NA enhances I_h through an increase in the sensitivity of the underlying channels to the voltage difference across the membrane. Such an increase in voltage sensitivity may result

from activation of a cyclic AMP-dependent protein kinase which alters the voltage-sensing portion of I_h channels. However, alternative explanations are possible and these mechanisms will only be revealed through detailed biophysical and biochemical experiments. In any case, the NA- and 5-HT-induced change of the voltage dependence of I_h activation bears important consequences for the function of thalamocortical relay neurones. The positive shift of I_h activation will increase the amount of h-current that is active at rest, leading to a depolarization of the cell towards the reversal potential of I_h, i.e. closer to firing threshold. Although the steepness of the activation curve ensures that even small changes in the voltage dependence of G_h will result in large changes in the amount active at resting membrane potentials, the amplitude of the resulting depolarization is small due to the close proximity of the normal resting potential (-60 to -70 mV) and the reversal potential of I_h (around -43 mV). In addition, enhancement of G_h at rest will have larger effects on the slow components of hyperpolarizing *versus* depolarizing inputs. Hyperpolarizing inputs (such as GABA-mediated increases in potassium conductance; Hirsch & Burnod, 1987; Crunelli, Haby, Jassik-Gerschenfeld, Leresche & Pirchio, 1988) will increase the driving force on I_h and therefore will be partially offset by compensatory movements of Na^+ and K^+ through the membrane. The enhancement of G_h at rest, furthermore, will insure that hyperpolarizing inputs of all durations will be decreased (e.g. Fig. 1*E*). The resulting reduction of hyperpolarizing inputs will have important implications for rhythmic burst firing *in vivo*, since some forms of activity, e.g. spindle waves, appear to depend upon the generation of Ca^{2+}-mediated rebound burst discharges activated by IPSPs (Steriade & Deschênes, 1984; Steriade & Llinás, 1988). Reduction in the amplitude of IPSPs through enhancement of I_h therefore may result in a corresponding reduction in this type of oscillation.

In contrast to the potent modulatory actions of enhancement of I_h at resting membrane potentials, increases of I_h while the thalamic neurone is depolarized into the single-spike firing mode will have distinctly different effects. Since single-spike firing threshold (approximately -55 mV) is largely out of the range of G_h (Fig. 9), enhancement of this conductance with NA or 5-HT will result in little or no change in membrane potential. However, upon hyperpolarization, the rate of repolarization of the membrane will be enhanced, thereby limiting prolonged hyperpolarizations.

Possible functional role of NA and 5-HT in the ascending control of arousal

Thalamic neurones display two prominent modes of action potential generation which vary with the behavioural state and which determine, in part, certain distinct features of the electroencephalogram. These two states are rhythmic burst firing which occurs during drowsiness and slow-wave sleep and tonic single-spike firing which occurs during periods of wakefulness and arousal (McCarley, Benoit & Barrioneuvo, 1983; Steriade & Deschênes, 1984). Rhythmic burst firing appears to occur in two distinct patterns: slow (1–2 Hz) rhythmic bursting and as spindle waves. Spindle waves appear in the electroencephalogram as 8–12 Hz oscillations which first increase and then decrease in amplitude and which originate within the thalamus (Steriade & Deschênes, 1984). While slow rhythmic burst firing appears to be an endogenous property of some thalamocortical relay neurones in the cat (McCormick & Prince, 1988; Haby *et al.* 1988; McCormick & Pape, 1990) spindle

wave generation appears to involve the synaptic and electrophysiological interaction of GABAergic cells of the nucleus reticularis with thalamocortical relay neurones of the specific relay nuclei (Steriade & Llinás, 1988). For example, intracellular recordings *in vivo* reveal that during the generation of a spindle wave, thalamocortical relay neurones receive a rhythmic barrage of IPSPs, a subset of which are followed by the generation of a low-threshold Ca^{2+} spike (Steriade & Deschênes, 1984). In this manner, the IPSP appears to provide the membrane hyperpolarization necessary for de-inactivation of the low-voltage-activated Ca^{2+} current. Once the low-threshold Ca^{2+} current is de-inactivated, the repolarizing phase of the IPSP can then activate this current and lead to a slow Ca^{2+} spike. Alteration in the amplitude of the synaptically mediated IPSP could, therefore, strongly influence this 8–12 Hz oscillation. In particular, the ability of NA and 5-HT to increase the amplitude and rate of activation of I_h may allow these neurotransmitters to selectively limit the amplitude and duration of membrane hyperpolarization and thus selectively dampen rhythmic burst activity in thalamocortical relay neurones during spindle wave generation. Although we have not yet tested this hypothesis directly on spindle waves, our observations on the ability of serotonergic and β-adrenergic receptor stimulation to limit the ability of thalamic neurones to respond to relatively short duration hyperpolarizing current pulses are consistent with this hypothesis.

The slow 1–2 Hz rhythmic burst firing in cat LGND relay neurones appears to depend critically upon the amplitude, voltage dependence and kinetics of activation and de-activation of I_h (McCormick & Pape, 1990). Indeed, either reduction or enhancement of this current with extracellular Cs^+ or isoprenaline/5-HT, respectively, can result in an abolition of this rhythm. Since the depolarization caused by I_h activation appears to be critical for triggering a low-threshold Ca^{2+} spike, severe reductions of I_h will tend to abolish slow rhythmic oscillations by blocking this depolarizing 'pacemaker' potential (e.g. Fig. 11 in McCormick & Pape, 1990). In contrast, an increase in I_h through a positive shift in the activation curve may keep the neurone depolarized out of the range of de-inactivation of the low-voltage-activated Ca^{2+} current, I_t, such that the Ca^{2+} spike cannot support rhythmic burst firing. Two hypotheses may be proposed from these data. First, the subset of thalamocortical relay neurones which exhibit 1–2 Hz spontaneous bursting in the cat LGND may represent those cells which possess a balance of I_h and the low-threshold Ca^{2+} current, I_t, which is conducive to this type of activity. Second, the positive shift of the I_h activation curve through activation of monoaminergic receptors may serve as a brake, that effectively interrupts slow oscillations in the thalamus. Interestingly, in the heart, modulation of the equivalent pacemaker current, I_f, through catecholamines (Brown, DiFrancesco & Noble, 1979; DiFrancesco, 1986; DiFrancesco, Ferroni, Mazzanti & Tromba, 1986) and acetylcholine (DiFrancesco & Tromba, 1987, 1988*a*, *b*) serves to control the rate of pacemaking and thus the frequency of rhythmic activity rather than to interrupt the rhythm. The possibility that weak stimulation of β-adrenergic or serotonergic receptors may increase the rate of rhythmic burst firing in thalamocortical relay cells has not yet been tested. However, the finding that partial suppression of I_h with Cs^+ may slow down the rate of rhythmic burst firing is supportive of this hypothesis (McCormick & Pape, 1990).

These results and those of previous investigations (reviewed by McCormick, 1989)

allow us to propose the following scenario of noradrenergic and serotonergic influences during increases in arousal and awakening from sleep. Extracellular single-unit recordings from both the source of noradrenergic input to the thalamus, the locus coeruleus, and the serotonergic input, the median raphe, have revealed the discharge rate of these neurones to vary in a state-dependent manner such that awakening from sleep or increases in behavioural arousal and attentiveness are associated with increases in firing rate of noradrenergic and serotonergic neurones (Trulson & Jacobs, 1979; Aston-Jones & Bloom, 1981; Jacobs, 1986). The subsequent increase in release of NA and 5-HT will have numerous and complex actions on its target neurones. In the thalamus, activation of β-adrenoceptors and serotonergic receptors will result in a positive shift of the I_h activation curve, and thereby dampening neuronal oscillations, as described above. In addition, activation of α_1-adrenoceptors by NA will reduce a resting potassium conductance and therefore depolarize the relay neurones and bring their membrane potential closer to single-spike firing threshold (McCormick & Prince, 1988). The subsequent inhibition of rhythmic burst firing and facilitation of single-spike activity should greatly facilitate the transfer to the cerebral cortex of incoming phasic EPSPs from stimulation of sensory receptive fields (Coenen & Vendrik, 1972; Livingstone & Hubel, 1981; McCormick & Feeser, 1990).

Once the PSPs reach the cerebral cortex, many of the cortical pyramidal cells will also be more responsive due to a decrease in spike frequency adaptation from block of the current underlying the slow after-hyperpolarization (I_{AHP}) by NA, 5-HT and other putative neurotransmitters and the M-current by 5-HT and acetylcholine (McCormick & Williamson, 1989). In this manner, we would like to propose that increases in the firing rate of noradrenergic and serotonergic neurones in the brain stem may modulate the pattern of thalamocortical activity from a state of rhythmic oscillation and unresponsiveness to peripheral inputs to a state of single-spike activity which is conducive, and indeed necessary for, cognition.

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REFERENCES

ASTON-JONES, G. & BLOOM, F. E. (1981). Activity of NE-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep–waking cycle. *Journal of Neuroscience* **1**, 876–886.

BOBKER, D. H. & WILLIAMS, J. T. (1989). Serotonin augments the cation current I_h in central neurons. *Neuron* **2**, 1535–1540.

BROWN, H. F., DIFRANCESCO, D. & NOBLE, S. J. (1979). How does adrenaline accelerate the heart? *Nature* **280**, 235–236.

COENEN, A. M. L. & VENDRIK, A. J. H. (1972). Determination of the transfer ratio of cat's geniculate neurons through quasi-intracellular recordings and the relation with the level of arousal. *Experimental Brain Research* **14**, 227–242.

COULTER, D. A., HUGUENARD, J. R. & PRINCE, D. A. (1989). Calcium currents in rat thalamocortical relay neurones: kinetic properties of the transient, low-threshold current. *Journal of Physiology* **414**, 587–604.

CRUNELLI, V., HABY, M., JASSIK-GERSCHENFELD, D., LERESCHE, N. & PIRCHIO, M. (1988). Cl^-- and K^+-dependent inhibitory postsynaptic potentials evoked by interneurones of the rat lateral geniculate nucleus. *Journal of Physiology* **399**, 153–176.

CRUNELLI, V., LIGHTOWLER, S. & POLLARD, C. E. (1989). A T-type Ca^{2+} current underlies low-threshold Ca^{2+} potentials in cells of the cat and rat lateral geniculate nucleus. *Journal of Physiology* **413**, 543–561.

DE LIMA, A. D. & SINGER, W. (1987). The brainstem projection to the lateral geniculate nucleus in the cat: Identification of cholinergic and monoaminergic elements. *Journal of Comparative Neurology* **259**, 92–121.

DIFRANCESCO, D. (1985). The cardiac hyperpolarizing-activated current, i_f, origins and developments. *Progress in Biophysics and Molecular Biology* **46**, 163–183.

DIFRANCESCO, D. (1986). Characterization of single pacemaker channels in cardiac sino-atrial node cells. *Nature* **324**, 470–473.

DIFRANCESCO, D., DUCOURET, P. & ROBINSON, R. B. (1989). Muscarinic modulation of cardiac rate at low acetylcholine concentrations. *Science* **243**, 669–671.

DIFRANCESCO, D., FERRONI, A., MAZZANTI, M. & TROMBA, C. (1986). Properties of the hyperpolarizing-activated current (i_f) in cells isolated from the rabbit sino-atrial node. *Journal of Physiology* **377**, 61–88.

DIFRANCESCO, D. & TROMBA, C. (1987). Acetylcholine inhibits activation of the cardiac pacemaker current, i_f. *Pflügers Archiv* **410**, 139–142.

DIFRANCESCO, D. & TROMBA, C. (1988*a*). Inhibition of the hyperpolarization-activated current (i_f) induced by acetylcholine in rabbit sino-atrial node myocytes. *Journal of Physiology* **405**, 477–491.

DIFRANCESCO, D. & TROMBA, C. (1988*b*). Muscarinic control of the hyperpolarization-activated current (i_f) in rabbit sino-atrial node myocytes. *Journal of Physiology* **405**, 493–510.

FAYOLLE, C., FILLION, M.-P., BARONE, P., OUDAR, P., ROUSSELLE, J.-C. & FILLION, G. (1988). 5-Hydroxytryptamine stimulates two distinct adenylate cyclase activities in rat brain: High-affinity activation is related to a 5-HT_1 subtype different from 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C}. *Fundamentals of Clinical Pharmacology* **2**, 195–214.

HABY, M., LERESCHE, N., JASSIK-GERSCHENFELD, D., SOLTESZ, I. & CRUNELLI, V. (1988). Spontaneous rhythmic depolarization in the principal cells of the lateral geniculate body in vitro: the role of NMDA receptors. *Compte rendu hebdomadaire des séances de l'Académie des sciences, Series III* **306**, 195–199.

HAGIWARA, N. & IRISAWA, H. (1989). Modulation by intracellular Ca^{2+} of the hyperpolarization-activated inward current in rabbit single sino-atrial node cells. *Journal of Physiology* **409**, 121–141.

HERNÁNDEZ-CRUZ, A. & PAPE, H.-C. (1989). Identification of two calcium currents in acutely dissociated neurons from the rat lateral geniculate nucleus. *Journal of Neurophysiology* **61**, 1270–1283.

HIRSCH, J. C. & BURNOD, Y. (1987). A synaptically evoked late hyperpolarization in the rat dorsal lateral geniculate nucleus in vitro. *Neuroscience* **23**, 457–468.

HOSHI, T., GARBER, S. S. & ALDRICH, R. W. (1988). Effect of forskolin on voltage-gated K^+ channels is independent of adenylate cyclase activation. *Science* **240**, 1652–1655.

HUGHES, I. E. & SMITH, J. A. (1978). The stability of noradrenaline in physiological saline solutions. *Journal of Pharmacy and Pharmacology* **30**, 124–125.

JACOBS, B. L. (1986). Single unit activity of locus coeruleus neurons in behaving animals. *Progress in Neurobiology* **27**, 183–194.

JAHNSEN, H. & LLINÁS, R. (1984*a*). Electrophysiological properties of guinea-pig thalamic neurones: an *in vitro* study. *Journal of Physiology* **349**, 105–226.

JAHNSEN, H. & LLINÁS, R. (1984*b*). Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurones *in vitro*. *Journal of Physiology* **349**, 227–247.

JONES, E. G. (1985). *The Thalamus*. Plenum Press, New York.

JONES, L. S., GAUGER, L. L. & DAVIS, J. N. (1985). Anatomy of brain alpha$_1$-adrenergic receptors: In vitro autoradiography with [^{125}I]-HEAT. *Journal of Comparative Neurology* **231**, 190–208.

JOUVET, M. (1972). The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep–waking cycle. *Ergebnisse der Physiologie, Biologischen, Chemie und experimentellen Pharmakologie* **64**, 166–307.

KUPFERMAN, I. (1980). Role of cyclic nucleotides in excitable cells. *Annual Review of Physiology* **42**, 629–641.

LAMARRE, Y., FILLION, M. & CORDEAU, J. P. (1971). Neuronal discharge of the ventrolateral nucleus of the thalamus during sleep and wakefulness in the cat. I. Spontaneous activity. *Experimental Brain Research* **12**, 480–498.

LINDVALL, I., BJÖRKLUND, A., NOBIN, A. & STENEVI, U. (1974). The adrenergic innervation of the rat thalamus as revealed by the glyoxylic acid fluorescence method. *Journal of Comparative Neurology* **154**, 317–348.

LIVINGSTONE, M. S. & HUBEL, D. H. (1981). Effects of sleep and arousal on the processing of visual information in the cat. *Nature* **291**, 554–561.

LLINÁS, R. R. (1988). The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. *Science* **242**, 1654–1664.

MCCARLEY, R. W., BENOIT, O. & BARRIONUEVO, G. (1983). Lateral geniculate nucleus unitary discharge in sleep and waking: State- and rate-specific aspects. *Journal of Neurophysiology* **50**, 798–818.

MCCORMICK, D. A. (1989). Cholinergic and noradrenergic modulation of thalamocortical processing. *Trends in Neurosciences* **12**, 215–221.

MCCORMICK, D. A. & FEESER, H. R. (1990). Functional implications of burst firing and single spike activity in lateral geniculate relay neurons. *Neuroscience* (in the Press).

MCCORMICK, D. A. & PAPE, H.-C. (1990). Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. *Journal of Physiology* **431**, 291–318.

MCCORMICK, D. A. & PRINCE, D. A. (1988). Noradrenergic modulation of firing pattern in guinea pig and cat thalamic neurons, in vitro. *Journal of Neurophysiology* **59**, 978–996.

MCCORMICK, D. A. & WILLIAMSON, A. (1989). Convergence and divergence of neurotransmitter action in human cerebral cortex. *Proceedings of the National Academy of Sciences of the USA* **86**, 8098–8102.

MORRISON, J. H. & FOOTE, S. L. (1986). Noradrenergic and serotonergic innervation of cortical, thalamic, and tectal visual structures in old and new world monkeys. *Journal of Comparative Neurology* **43**, 117–138.

PAPE, H.-C. & MCCORMICK, D. A. (1989). Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current. *Nature* **340**, 715–718.

PAZOS, A., CORTES, R. & PALACIOS, J. M. (1985). Quantitative autoradiographic mapping of serotonin receptors in rat brain. II. Serotonin-2 receptors. *Brain Research* **346**, 231–249.

PAZOS, A. & PALACIOS, J. M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Research* **346**, 205–230.

PEROUTKA, S. J. (1988). 5-Hydroxytryptamine receptor subtypes. *Annual Reviews of Neuroscience* **11**, 45–60.

SEAMON, K. B. & DALY, J. W. (1986). Forskolin: Its biological and chemical properties. *Advances in Cyclic Nucleotide and Protein Phosphorylation Research* **20**, 1–150.

STERIADE, M. & DESCHÊNES, M. (1984). The thalamus as a neuronal oscillator. *Brain Research Reviews* **8**, 1–63.

STERIADE, M., DESCHÊNES, M., DOMICH, L. & MULLE, C. (1985). Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. *Journal of Neurophysiology* **54**, 1473–1497.

STERIADE, M. & LLINÁS, R. (1988). The functional states of the thalamus and the associated neuronal interplay. *Physiological Reviews* **68**, 649–742.

TOKIMASA, T. & AKASU, T. (1990). Cyclic AMP regulates an inward rectifying sodium–potassium current in dissociated bull-frog sympathetic neurones. *Journal of Physiology* **420**, 409–429.

TRULSON, M. E. & JACOBS, B. L. (1979). Raphe unit activity in freely moving cats: Correlation with level of behavioural arousal. *Brain Research* **163**, 135–150.

WAGONER, P. K. & PALLOTA, B. S. (1988). Modulation of acetylcholine receptor desensitization by forskolin is independent of cAMP. *Science* **240**, 1655–1657.