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SEROTONIN AND NORADRENALINE EXCITE GABAergic NEURONES OF THE GUINEA-PIG AND CAT NUCLEUS RETICULARIS THALAMI

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SUMMARY

1. The actions of serotonin (5-HT) and noradrenaline (NA) in the cat perigeniculate nucleus (PGN) and the guinea-pig nucleus reticularis thalami (NRT) were investigated with extracellular and intracellular recordings obtained from neurones in thalamic slices maintained *in vitro*.

2. Single, local application of either 5-HT or NA resulted in pronounced (5–50 Hz) and prolonged (2–10 min) excitation associated with the occurrence of single-spike activity. Serotoninergic excitation was specifically blocked by the 5-HT_2/5-HT_{1C} antagonists ketanserin and ritanserin, but not by the 5-HT_{1A} antagonist pindolol or the 5-HT_3 antagonist ICS 205–930. Furthermore, the 5-HT response was mimicked by α-methyl-5-HT, but not by the 5-HT_{1A} agonist 8-hydroxy-dipropylaminotetralin (8-OHDPAT) or the 5-HT_3 agonist 2-methyl-5-HT. Together, these results indicate that this excitatory response is mediated through 5-HT_2 receptors with the possible involvement of 5-HT_{1C} receptors.

3. Noradrenergic excitation was specifically blocked by the α_1-antagonist prazosin, but not by the β-antagonist propranolol or the α_2-antagonist yohimbine. Similarly, the response was mimicked by the α-agonist phenylephrine, but not by the β-agonist isoprenaline. These results indicate that the noradrenergic excitation is mediated by α_1-adrenoceptors.

4. Block of synaptic transmission either by lowering external calcium concentration ($[Ca^{2+}]_o$) to 0·5 mM and raising external magnesium concentration ($[Mg^{2+}]_o$) to 10 mM or by local application of tetrodotoxin failed to block the excitatory or depolarizing response to 5-HT or NA indicating that these responses are direct and not mediated through the release of other neurotransmitters.

5. Intracellular recordings revealed that the 5-HT- and NA-induced excitations are mediated by a pronounced slow depolarization associated with an apparent decrease in input conductance and an increase in the membrane time constant. Current *versus* voltage plots obtained under voltage clamp before and during the presence of 5-HT and NA revealed that these neurotransmitters induced an inward current which reversed to an outward current at -107 and -110 mV, respectively, in 2·5 mM external potassium concentration ($[K^+]_o$). This reversal potential was identical to that associated with an increase in potassium conductance activated by

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acetylcholine (−110 mV) in the same neurones. Plots of the amplitude of the 5-HT- or NA-induced current *versus* membrane potential revealed a linear relationship in the voltage range from −140 to −60 mV. These results indicate that 5-HT and NA excite NRT and PGN cells through a decrease in a 'leak' potassium current which we term here I_{KL}.

6. The 5-HT- and NA-induced slow depolarizations were non-additive in that a maximal application of one reduced or blocked the response to the other. This result suggests that these two neurotransmitters are converging onto the same potassium conductance.

7. Extracellular and intracellular recordings revealed that the 5-HT- or NA-induced slow depolarization potently inhibits burst firing and promotes the occurrence of single-spike activity, although in some neurones the probability of rhythmic bursts is enhanced during the final stages of the slow excitation. We suggest that the switch in firing mode from rhythmic oscillation to single-spike activity observed in the NRT and PGN during increases in arousal and attentiveness is mediated in part by a depolarization resulting from increased release of 5-HT and NA. Likewise, decreases in release of these two neurotransmitters may promote the occurrence of rhythmic oscillations, such as spindle waves, during periods of drowsiness, inattentiveness, or slow-wave sleep.

INTRODUCTION

The γ-aminobutyric acid (GABAergic) neurones in the mammalian thalamus form two distinct subgroups: those within thalamic relay nuclei and those within the nucleus reticularis (NRT; Jones, 1985). The nucleus reticularis is a shell-shaped collection of GABAergic neurones which surrounds much of the dorsal and lateral extent of the thalamus and which is interposed within the thalamocortical and corticothalamic fibres that connect the thalamus and cerebral cortex (Houser, Vaughn, Barber, & Roberts, 1980; Jones, 1985). The portion of the NRT which is adjacent to the dorsal lateral geniculate nucleus in the cat is known as the perigeniculate nucleus (PGN; Fitzpatrick, Penny & Schmechel, 1984). The two major afferents to the NRT arise from collaterals of the thalamocortical and corticothalamic fibres, while the major efferent of this nucleus is a direct projection back to the relay neurones and interneurones of the thalamic relay nuclei (Steriade, Parent & Hada, 1984; reviewed in Steriade & Deschênes, 1984; Steriade & Llinás, 1988). Therefore, the NRT forms feedforward (corticothalamic) and feedback (thalamocortical) inhibitory pathways through which the pattern of thalamocortical activity can be markedly influenced (Steriade & Llinás, 1988).

Neuronal activity in the NRT displays two distinct states: rhythmic generation of high-frequency bursts of action potentials, and single-spike (also known as tonic) activity (reviewed in Steriade & Deschênes, 1984; Steriade & Llinás, 1988). Which of these two types of activity NRT neurones generate depends largely upon the behavioural state of the animal. During periods of slow-wave sleep, inattentiveness, or drowsiness, NRT neurones tend to fire in rhythmic bursts while during periods of arousal and attentiveness, NRT neurones generate tonic activity characterized by trains of single action potentials (Steriade, Domich & Oakson, 1986; Shosaku, Kayama, Sumitomo, Sugitani & Iwama, 1989). Rhythmic burst generation during

slow-wave sleep tends to occur as a 1–2 s duration clustering of high-frequency bursts with an interburst interval of 7–14 Hz and underlie the so-called spindle waves of the electroencephalogram (Steriade & Deschênes, 1984; Steriade & Llinás, 1988). The ability of NRT neurones to generate these two distinct types of neuronal activity results from the presence of a strong low-threshold Ca^{2+} current, also known as the T-current (Jahnsen & Llinás, 1984*a*, *b*; Avanzini, de Curtis, Panzica & Spreafico, 1989). Intracellular recordings from NRT neurones *in vivo* and *in vitro* have revealed that at membrane potentials negative to approximately −60 mV, these neurones tend to generate rhythmic bursts of action potentials characterized by the rhythmic occurrence of low-threshold Ca^{2+} spikes. Depolarization of the neurone to membrane potentials positive to −60 mV abolishes this type of activity and replaces it with trains of single action potentials (McCormick & Prince, 1986; Mulle, Madariaga & Deschênes, 1986; Avanzini *et al.* 1989). This voltage dependence of the pattern of neuronal activity generated results directly from the voltage dependence of the low-threshold Ca^{2+} current (Jahnsen & Llinás, 1984*a*, *b*; Avanzini *et al.* 1989).

The depolarization which characterizes the shift from rhythmic oscillation to single-spike activity in the NRT *in vivo* presumably arises in response to some change in afferent activity. Although increased excitation from corticothalamic and thalamocortical collaterals are important in the determination of the pattern of activity generated in the NRT, it is the inputs from the brainstem and basal forebrain that are believed to control the long term changes in firing mode of these neurones (see Steriade & Deschênes, 1984; Steriade & Llinás, 1988). Retrograde, anterograde and immunohistochemical tracing techniques have revealed the NRT to be innervated by cholinergic fibres from the nucleus basalis of the basal forebrain and from the pedunculopontine and lateral dorsal tegmental nuclei of the brainstem, by noradrenergic fibres from the locus coeruleus, and by serotoninergic fibres from the dorsal raphe (Cropper, Eisenman & Azmitia, 1984; Morrison & Foote, 1986; de Lima & Singer, 1987; Levey, Hallanger & Wainer, 1987; Steriade, Parent, Pare & Smith, 1987*b*; Wilson & Hendrickson, 1988). Activation of cholinergic inputs to the NRT results in disruption of on-going rhythmic oscillations, as well as inhibition of single-spike activity (Steriade & Llinás, 1988; Hu, Steriade & Deschênes, 1989). In contrast, activation of presumed noradrenergic inputs can promote the occurrence of single-spike activity, although the effects of this activation on rhythmic oscillation have not been well characterized (Kayama, Negi, Sugitani & Iwama, 1982). The effects of activation of serotoninergic inputs to the NRT have not been studied in detail, although one report indicated that electrical stimulation of the dorsal raphe inhibits burst firing in NRT neurones (see Fig. 1 in Yoshida, Sasa, & Takaori, 1984).

Intracellular investigations of the actions of these neurotransmitters have revealed that activation of presumed cholinergic inputs or application of acetylcholine result in a marked hyperpolarization of NRT neurones through an increase in a potassium conductance (McCormick & Prince, 1986; Hu *et al.* 1989). In contrast, activation of presumed noradrenergic inputs, or application of noradrenaline (NA), depolarizes NRT neurones (Kayama *et al.* 1982; McCormick & Prince, 1988). Although the ionic mechanisms of this depolarization were not known, the similarity of this response to the depolarizing response of dorsal lateral geniculate (LGND) relay neurones to NA, which is known to be mediated by a decrease in a potassium conductance (McCormick & Prince, 1988), suggested a similar mechanism. These results suggest

that the brainstem noradrenergic, and perhaps serotoninergic, inputs may contribute greatly to the shift of NRT neurones from rhythmic oscillation to single-spike activity during increases in arousal and attentiveness. Here we examine the postsynaptic actions, and the consequences of these actions, of NA and 5-hydroxytryptamine (5-HT) on neurones of the NRT.

METHODS

Methods for preparation of thalamic slices and recording from PGN and NRT neurones are similar to those published previously (McCormick & Prince, 1986; McCormick & Pape, 1990). Male or female adult, Hartley guinea-pigs were deeply anaesthetized with sodium pentobarbitone (35 mg kg^{-1} I.P.) and killed by decapitation. One adult cat was pre-anaesthetized with ketamine (25 mg kg^{-1} I.M.), very deeply anaesthetized with sodium pentobarbitone (25–35 mg kg^{-1} I.V.) and killed by decapitation. In both cases, the region of brain containing either the reticular thalamic nucleus or the perigeniculate nucleus was removed, placed in cold (5 °C) bathing solution, and sectioned as 400 μm thick slices on a Vibratome (Ted Pella Inc., Tustin, CA). Thalamic slices were placed in an interface-style recording chamber (Fine Science Tools, Foster City, CA) and allowed to recover for at least 2 h before recording commenced. During bath application of agonists and antagonists, the recording well was modified to a semi-submerged preparation by suspending the tissue between two nylon nets and raising the bathing medium so as to just fill the top net. This arrangement allowed a much faster flow rate (3–5 ml min^{-1}) and presumably an increased control of the extracellular milieu. The bathing medium contained (in mM): NaCl, 126; KCl, 2·5; $MgSO_4$, 1·2; Na_2HPO_4, 1·25; $CaCl_2$, 2; $NaHCO_3$, 26; dextrose, 10; and was aerated with 95% O_2–5% CO_2 to a final pH of 7·4.

Agonists were dissolved in the bathing medium and were generally applied with the pressure-pulse technique in which a brief pulse of pressure (10–100 ms; 200–350 kPa) to a broken micropipette (tip diameter 2–10 μm) was used to extrude volumes of 2–20 pl. Antagonists were typically applied through addition to the bathing medium. The drugs α-methyl-5-HT, 2-methyl-5-HT, 8-OHDPAT, and ICS 205-930 were obtained from Research Biochemicals Incorporated (Natwick, MA). Ritanserin was a gift from Janssen (Beerse, Belgium) while pindolol was a gift from Sandoz Research Institute (East Hanover, NJ). All other drugs were obtained from Sigma Chemical Co. (St. Louis, MO). Extracellular single-unit recordings were obtained with tungsten microelectrodes (Frederick Haer Corporation, Brunswick, Maine), while intracellular recordings were obtained with microelectrodes formed on a Sutter Instruments P-80/PC micropipette puller, filled with 4 M-potassium acetate, and bevelled to a final resistance of 60–80 MΩ. Bevelled electrodes with a relatively high resistance were found to be necessary to obtain high quality intracellular recordings from NRT cells without substantial damage to the neurone. Lower-resistance electrodes formed from thin-wall glass, which we have used previously to obtain high quality recordings from thalamic relay neurones or cortical pyramidal cells, only rarely yielded intracellular recordings in NRT neurones, and when they did, were always associated with evidence of substantial damage to the neurone (e.g. low input resistance and short time constant).

Single-electrode voltage clamp was performed with an Axoclamp-1A amplifier connected to an IBM AT computer operating PClamp software (Axon Instruments). Switching frequencies were generally 3–4 kHz and gain was between 0·2 and 0·5 nA mV^{-1}. During current clamp, bridge balance was continuously monitored and adjusted while during voltage clamp, head stage output was continuously monitored to ensure adequate settling time. The unusually high input resistance of NRT neurones (100–500 MΩ) facilitated the acquisition of sufficient voltage clamp by limiting the amplitude of currents required to move the membrane over a wide range of voltages. In addition, bevelling the microelectrodes greatly improved their current-passing capabilities. Current *versus* voltage (*I–V*) plots were obtained by steadily hyperpolarizing the neurones from a holding potential of −60 mV to between −120 and −140 mV over a period of 5–10 s and measuring the amount of current required to do so. From three to ten individual voltage clamp traces were averaged to reduce noise.

The perigeniculate nucleus was localized in the cat LGND slice as a thin band of cells just superficial to lamina A. Upon completion of recording, extracellular application of dye was used to mark each recording site. The slices were then fixed in phosphate-buffered paraformaldehyde (4%),

resectioned at 50 μm, and counter stained with Cresyl Violet. Examination of the tissue revealed that all of the recordings were within the thin band of cells lying just dorsal to lamina A of the cat lateral geniculate nucleus. The NRT of the guinea-pig was readily visible in the dissecting microscope or to the unaided eye during the course of the experiments. Immunohistochemical staining for the GABA synthetic enzyme glutamic acid decarboxylase has previously been performed to confirm our localization of this nucleus in this recording situation (McCormick & Prince, 1986).

Extracellular single-unit recordings were displayed on a chart-recorder (Gould Instruments) by conversion of the data to an average spike-frequency histogram through a comparator, counter and digital-to-analog converter (e.g. Fig. 1*A*). Each occurrence of an action potential is reflected as a step increment in pen position on the chart-recorder. After a pre-set time period (typically 2 s for local applications and 10 s for bath applications of agonists) the counter was reset. Therefore the height of each bin is representative of the average firing rate during that time period. This method of data display was sufficient for single-spike activity, but misrepresented the occurrence of high-frequency burst discharges. Therefore, to insure accuracy, we also illustrate portions of the original recordings for detail.

RESULTS

Extracellular single-unit recordings were obtained from ten neurones in the cat perigeniculate nucleus (PGN). These neurones were considered to be part of the PGN on the basis of their presence as a thin sheet of cells within the fibres of the optic radiation just dorsal to lamina A, their unusually short duration action potentials (see below), and their pronounced inhibition by acetylcholine (ACh) (Fig. 1*F*). Application of either 5-HT ($n = 7$; 300 μM in micropipette) or NA ($n = 4$; 500 μM in micropipette) resulted in a marked (up to 50 Hz) and prolonged (up to 20 min) excitation characterized by the presence of a steady increase and subsequent decrease back to baseline of single-spike activity (Fig. 1*A–E*). Although this was the typical response, other firing patterns were observed in various neurones (see also below). In three PGN cells, application of 5-HT or NA resulted in the appearance of one or more rhythmic bursts of action potentials (Fig. 1*D*) with each burst containing three to eight action potentials discharging at a rate of 300–500 Hz (Fig. 1*E*, right). These rhythmic bursts of action potentials could appear on the ascent of the excitatory response (Fig. 1*D*), but when they did occur, were usually much more pronounced during the final stages of the response (Fig. 1*E*). The interburst frequency was generally between 1 and 8 Hz, and the rhythmic bursts were often grouped together and could be followed by a short period of single-spike activity (Fig. 1*E*). Although both 5-HT and NA were potently excitatory, the response to 5-HT was in general longer in duration and larger in amplitude than the response to NA, even though the concentration of 5-HT in the micropipette was less than that of NA (Fig. 1). Interestingly, application of ACh (1–5 mM in micropipette) to all PGN neurones tested ($n = 7$) resulted in inhibition of single-spike activity, whether this activity was spontaneous or induced by NA or 5-HT (Fig. 1*F*). We found no evidence of either fast or slow excitatory responses to ACh. In contrast to the responses to NA and 5-HT, the response to ACh was both quick in onset (< 1 s) and short in duration (typically 1–4 s). The responses of PGN neurones to these three neurotransmitters were markedly different from those of presumed relay neurones in the neighbouring A lamina, where presumed relay cells are typically excited by ACh and NA, but not by 5-HT.

Extracellular single-unit recordings ($n = 49$) from neurones in the guinea-pig NRT

revealed responses to 5-HT, NA, and ACh identical to those found in the cat PGN, regardless of their anatomical location within the NRT. Application of either 5-HT or NA resulted in a pronounced and prolonged discharge of single-spike activity (Figs 2 and 3) while application of ACh was followed by inhibition of single-spike activity

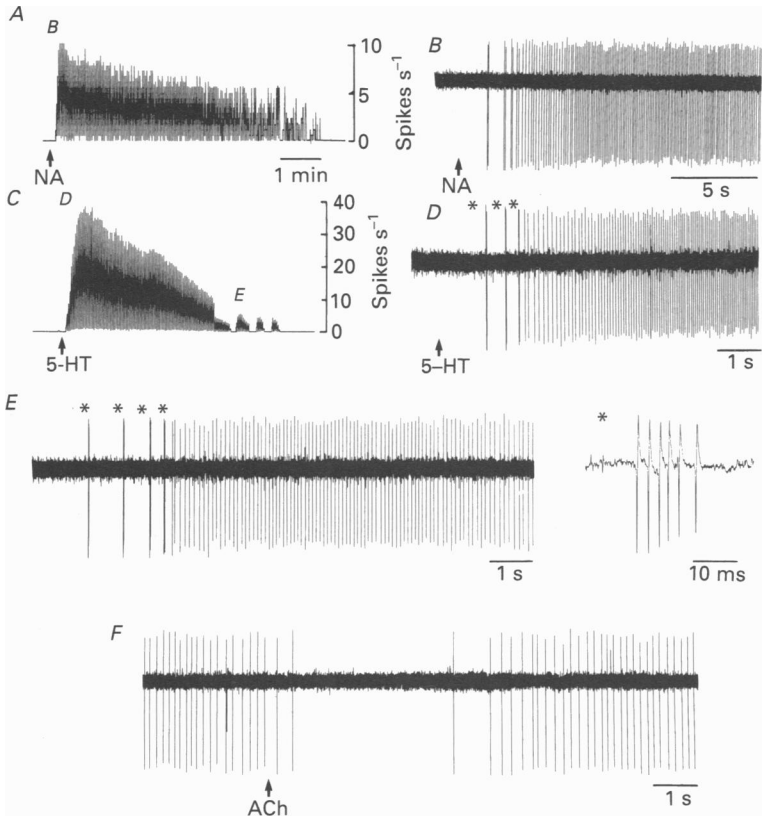

Fig. 1. Responses of cat perigeniculate neurones to application of NA, 5-HT and ACh. Application of either NA (*A*) or 5-HT (*C*) results in prolonged excitation, while application of ACh (2 mM in micropipette) results in a brief period of inhibition (*F*). Portions of the single-unit recordings corresponding to the portions of the histograms indicated are shown for detail in *B* and *D* and *E*. The initial response of the PGN neurone illustrated in *C* to 5-HT is marked by the rhythmic occurrence of three high-frequency burst discharges (*) followed by a single-spike activity which steadily increases in frequency (*D*). Near the end of the excitatory response, the neurone exhibited three sets of grouped discharge characterized by the rhythmic occurrence of two to four high-frequency burst discharges (*) followed by a few seconds of single-spike activity (*E*). An example of one high-frequency burst discharge is illustrated in *E*, right (*). *F*, application of ACh to a tonically active PGN neurone results in a brief period of inhibition with no evidence of fast or slow excitation. Sample period for histograms was 2 s bin^{-1}. In this and all figures the concentration of NA in the micropipette was 500 μM and the concentration of 5-HT was 300 μM.

without evidence for fast or slow excitation, as previously reported (McCormick & Prince, 1986). Over the course of these recordings, a hierarchy of firing patterns became apparent through which NRT neurones may transit during the 5-HT- or NA-induced excitation. The ascent of the response was as follows (see Figs 1–5):

Silent → (Rhythmic bursting → Intermittent single-spike firing) → Tonic single spike activity;

while the descent of the response was just the opposite:

Tonic single-spike activity → (Intermittent single spike firing → Rhythmic bursting) → Silent.

The parenthesis around rhythmic bursting and intermittent single-spike activity denote that these phases of activity did not always occur, but when they did, they appeared in the indicated positions of the hierarchy. In this manner, application of 5-HT or NA to a silent neurone could result in the appearance of rhythmic burst firing followed by tonic single-spike activity (Fig. 1*D*), or to intermittent firing (in which groups of two to twenty single action potentials were separated by a brief period of silence) followed by tonic single-spike activity (see Fig. 3*E* and *F*). Similarly, application of 5-HT or NA to a rhythmically bursting neurone resulted in intermittent or tonic single-spike activity (e.g. Fig. 5*A*), while application to a neurone already displaying single-spike activity always resulted in an increase in frequency of single-spike activity and never in the appearance of rhythmic bursts. The most typical response to NA or 5-HT was the activation of single-spike activity from a previously silent neurone (n = (34 out of a total of 49); Fig. 2*B*) with the other neurones also exhibiting single-spike activity in response to NA or 5-HT, but which was preceded or followed by the occurrence of rhythmic burst firing (e.g. Fig. 1*D* and *E*). Intracellular recordings revealed that these different patterns of activity are intrinsic to NRT neurones and are generated in response to simple membrane depolarization (see below).

Pharmacology of NA- and 5-HT-induced excitation

The pharmacological identity of the receptors mediating the excitatory responses to 5-HT and NA were investigated in the guinea-pig NRT. Bath applications of either 5-HT or NA resulted in an increase in single-spike activity with a concentration threshold of approximately 1–3 μM (not shown). This increase in single-spike activity persisted for as long as NA or 5-HT was present in the bath (tested for up to 1 h 30 min), indicating a lack of strong desensitization. The NA response was mimicked by local applications of the α-agonist phenylephrine (250 μM in micropipette; $n = 9$), but not by the β-agonist isoprenaline (250 μM in micropipette; $n = 3$; Fig. 2*A*, *B* and *C*). In addition, the NA response persisted in the presence of high concentrations of the β-antagonist propranolol (50 μM) and the α_2-antagonist yohimbine (10 μM) ($n = 3$; not shown) and was selectively and completely blocked by the α_1-antagonist prazosin (0·5 μM; $n = 4$) (Fig. 2*D*). In contrast, the excitatory response to 5-HT was not blocked by prazosin ($n = 9$; Fig. 2*D*) indicating that this response is not due to a cross-over of 5-HT onto α_1-adrenoceptors.

Previous investigators have reported excitatory responses to 5-HT in other regions of the nervous system in response to activation of 5-HT_{1A}, 5-HT_2, and 5-HT_3 receptors (e.g. North & Uchimura, 1989; Takahashi & Berger, 1990; Todorović & Anderson, 1990; reviewed by Bobker & Williams, 1990), therefore we addressed

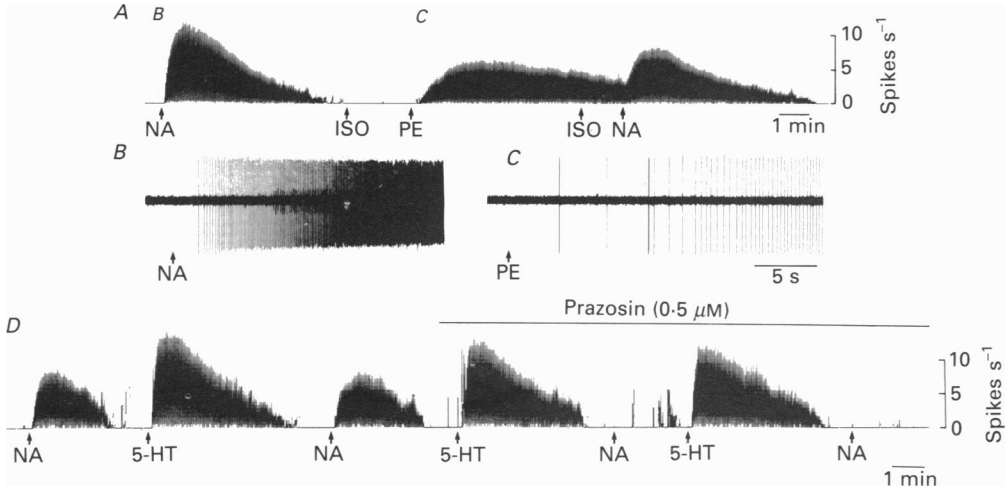

Fig. 2. Noradrenaline excites guinea-pig NRT cells through α_1-adrenoceptors. *A*, application of NA results in a pronounced slow excitation. Application of the β-agonist isoprenaline (ISO) does not excite the cell, while local application of the α-agonist phenylephrine (PE) does. Application of ISO during the PE-induced excitation still reveals no excitation, indicating that the lack of observance of excitatory responses to this agonist is not due to a subthreshold membrane potential. An additional application of NA illustrates the ability of the neurone to be further excited during the PE-induced response, indicating that the lack of response to ISO is not due to occlusion. *B* and *C*, illustration of portions of original recordings indicated in *A*. No rhythmic burst firing was present in this neurone. *D*, bath application of prazosin quickly and specifically blocks the excitatory response to NA, but not to 5-HT. Recordings for *A*, *B* and *C* from a different guinea-pig to those for *D*.

whether any of these receptors may be mediating the present response. Block of 5-HT_{1A} and 5-HT_{1B} receptors with pindolol (1 μM) did not block the response to 5-HT ($n = 3$; not shown). Similarly, bath application of the 5-HT_{1A} agonist 8-hydroxy-dipropylaminotetralin (8-OHDPAT; 1 μM) did not result in any response (Fig. 3*A*). Block of 5-HT_3 receptors with ICS 205–930 (1 μM) also did not block the response to 5-HT ($n = 3$; not shown) and bath application of the 5-HT_3 agonist 2-methyl-5-HT (1 μM) did not result in any response ($n = 4$; not shown). In contrast, bath application of α-methyl-5-HT (1–2·5 μM) resulted in pronounced excitatory responses ($n = 4$; Fig. 3*A* and *C*). α-Methyl-5-HT has previously been reported to be a preferential 5-HT_2 agonist (Engel, Gothert, Hoyer, Schlicker & Hillenbrand, 1986), although more recent results show that it also has substantial affinity for 5-$HT_{1A,1B,1Cand1D}$, but not 5-HT_{1E} or 5-HT_3 receptors (Ismaiel, Titeler, Miller, Smith & Glennon, 1990). To further investigate the possible involvement of 5-HT_2

receptors, we bath applied either the 5-HT_2 antagonist ketanserin (0·5 μM) or ritanserin (1 μM) while examining the response to 5-HT and NA. Repeated application of 5-HT and NA (2–6 times each) were performed in order to establish a steady baseline of responses prior to the application of either of these two

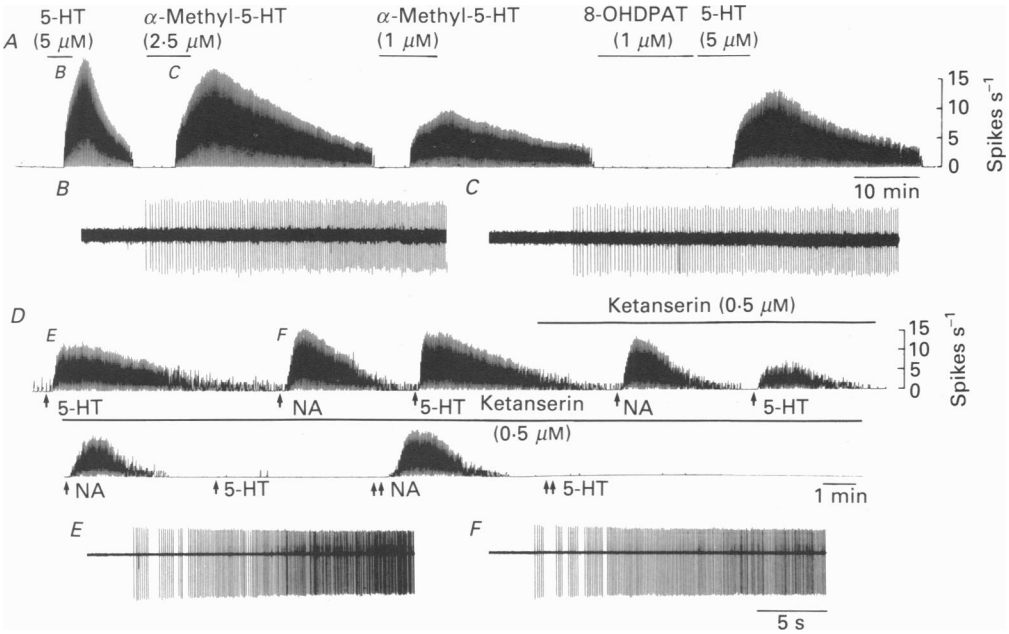

Fig. 3. Serotonin excites guinea-pig NRT cells through 5-HT_2 or 5-HT_{1C} receptors. Bath application of 5-HT (5 μM), or α-methyl-5-HT (1 or 2·5 μM), but not 8-OHDPAT (1 μM) results in pronounced excitation (*A*) characterized by the activation of single-spike activity (*B* and *C*). Bath application of the 5-HT_2 antagonist ketanserin (0·5 μM) abolishes the response to 5-HT, while that to NA is only partially reduced (*D*). Illustration of unit recording in *E* and *F* demonstrate the 5-HT and NA induction of intermittent- followed by tonic single-spike activity in this neurone. Bin width for the histograms in *A* was 10 s while in *D* it was 2 s.

antagonists. Ketanserin completely blocked the response to 5-HT and reduced the response to NA ($n = 4$; Fig. 3*D*). The reduction in the response to NA is expected given the substantial affinity of ketanserin for α_1-adrenoceptors (Leysen, Gommeren, Van Gompel, Wynants, Janssen & Laduron, 1985). Doubling of the application of NA was able to largely restore the excitatory response to this neurotransmitter, while doubling of the application of 5-HT still resulted in no response (Fig. 3*D*). Similar results were obtained with ritanserin, although this antagonist had less of a suppressant effect on the response to NA ($n = 5$; not shown). Prolonged wash-out of ketanserin or ritanserin was not able to restore the response to 5-HT, as previously reported for these concentrations of antagonists (e.g. North & Uchimura, 1989), and as expected by the slow dissociation constants for these drugs (Leysen *et al.* 1985). It is likely that the loss of the 5-HT response was specific to the application of

ketanserin or ritanserin, since the response to NA persisted in the presence of these drugs and we took care to examine only those cells which had not shown prior signs of response decrement to sequential applications of 5-HT in normal solution.

These results suggest that the 5-HT excitatory response is mediated by the 5-HT_2 subclass of receptor, although mediation or contribution by the 5-HT_{1C} subtype is

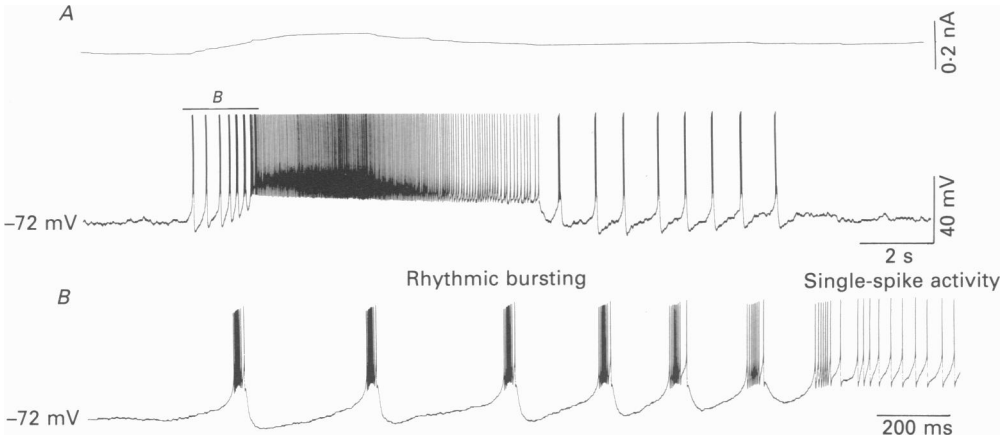

Fig. 4. Nucleus reticularis neurones display different modes of action potential generation depending upon membrane potential. At −72 mV. This NRT neurone is silent, while slight depolarization with the intracellular injection of current (top trace) results in the appearance of rhythmic high-frequency burst discharges (expanded in *B* for detail). Further depolarization increases the rate of burst generation and then abruptly changes the firing pattern to that of single-spike activity. Repolarization of the membrane by removal of the current injection results in reappearance of burst discharges followed once again by a lack of activity.

also possible since ketanserin and ritanserin both have substantial affinity for this subtype of receptor (Leysen *et al.* 1985).

Firing properties of NRT neurones and their alteration by 5-HT and NA

The cellular and ionic basis for the 5-HT- and NA-induced excitations were investigated with intracellular recordings from twenty-seven guinea-pig NRT neurones. Intracellularly, NRT cells were unusual in that they possessed high input resistances (from 70 to > 500 MΩ, average = 153 ± 93; S.D.), short duration (0·44–0·84 ms at base) action potentials, and tended to generate rhythmic bursts of action potentials upon removal of a hyperpolarizing current pulse as previously reported (McCormick & Prince, 1986; Avanzini *et al.* 1989). Intracellular recordings revealed that the various states of activity exhibited in response to NA or 5-HT are due to the intrinsic properties of NRT neurones, and perhaps to their local interconnections. These recordings revealed that the hierarchy of activity found with extracellular recordings arises from the value of the membrane potential such that steady depolarization of an NRT neurone from approximately −72 mV to approximately −52 mV results in first the appearance of rhythmic burst firing (Fig. 4*B*), followed by intermittent single-spike activity (not shown), followed by tonic

single-spike activity (Fig. 4). Rhythmic burst firing occurred at a frequency range of 1–8 Hz (typically 4–7 Hz) and appeared as a sequence of low-threshold Ca^{2+} spikes (Jahnsen & Llinás, 1984*a*, *b*) separated by an after-hyperpolarization or 'pacemaker' potential of unknown origin (Fig. 4*B*). We have previously demonstrated that similar 'pacemaker' potentials can arise either from the activation of a hyperpolarization-activated cation current in LGND relay neurones (McCormick & Pape, 1990), or from the activation of a Ca^{2+}-activated K^+ current in parataenial thalamic neurones (McCormick & Prince, 1988). Preliminary results indicate that NRT neurones possess little hyperpolarization-activated cation current, and therefore it is less likely that this current underlies this form of rhythmic burst firing in these neurones (D. A. McCormick, unpublished observations). Avanzini *et al.* (1989) have recently suggested that rhythmic burst firing in NRT neurones arises from an interaction of the low-threshold Ca^{2+} spike with a Ca^{2+}-activated K^+ current. In this manner, the frequency of burst firing would be determined by the amplitude of the underlying Ca^{2+} spike and subsequently the amplitude and duration of the resulting Ca^{2+}-activated K^+ current (e.g. Fig. 4*B*). The possible contribution of local synaptic interactions with other NRT neurones or relay neurones remain to be examined, although we have found that NRT neurones are capable of generating rhythmic Ca^{2+} spikes in the presence of the sodium channel poison tetrodotoxin, suggesting that these oscillations do not arise entirely from synaptic interactions (not shown).

Application of 5-HT or NA to NRT neurones during intracellular recording revealed a pronounced slow depolarization in response to these two agonists (Figs 5 and 6). This slow depolarization changed the firing mode of NRT neurones along the hierarchy described for extracellular recordings. Thus, application of 5-HT to a rhythmically bursting neurone resulted in an abolition of this activity and the appearance of single-spike firing (Fig. 5). Compensation for the 5-HT-induced depolarization with the intracellular injection of inward current (Fig. 5, −DC) completely reversed this effect, indicating that it arises largely from depolarization of the membrane. Application of 5-HT or NA to neurones which were silent at rest resulted in a substantial (5–20 mV) depolarization which led to the generation of single-spike activity (e.g. Figs 6 and 8). One or more rhythmic bursts of action potential could appear on the ascent of this response if the rate of depolarization was great enough to activate the low-threshold Ca^{2+} spike and if the initial membrane potential was between approximately −75 and −65 mV (not shown; see Fig. 4). On the descent of the depolarizing response, rhythmic oscillation could also occur, and appeared similar to that which is endogenous to NRT neurones in the membrane potential range of −75 and −60 mV (e.g. see Figs 4 and 5).

The slow depolarizing or excitatory responses to 5-HT and NA appeared to be due to direct stimulation of postsynaptic receptors, since these responses were not blocked when synaptic transmission was blocked either through the bath application of low Ca^{2+} (0·5 mM) and elevated Mg^{2+} (10 mM) and added Ni^{2+} (0·5 mM) ($n = 3$) or through the local application of the Na^+ channel poison tetrodotoxin ($n = 4$; cf. Fig. 5*A* and *B*). However, the low Ca^{2+}-, elevated Mg^{2+}- and Ni^{2+}-containing solution did abolish the occurrence of high-frequency burst activity in response to NA or 5-HT (not shown), confirming an important role of Ca^{2+} entry on this feature of NRT electroresponsiveness (Avanzini *et al.* 1989).

The functional consequences of the change in firing mode from one of rhythmic burst generation to single-spike activity were investigated by examining the response of NRT neurones to activation of excitatory postsynaptic potentials (EPSPs) through electrical single-shock stimulation of fibres lateral to the NRT ($n = 4$). In

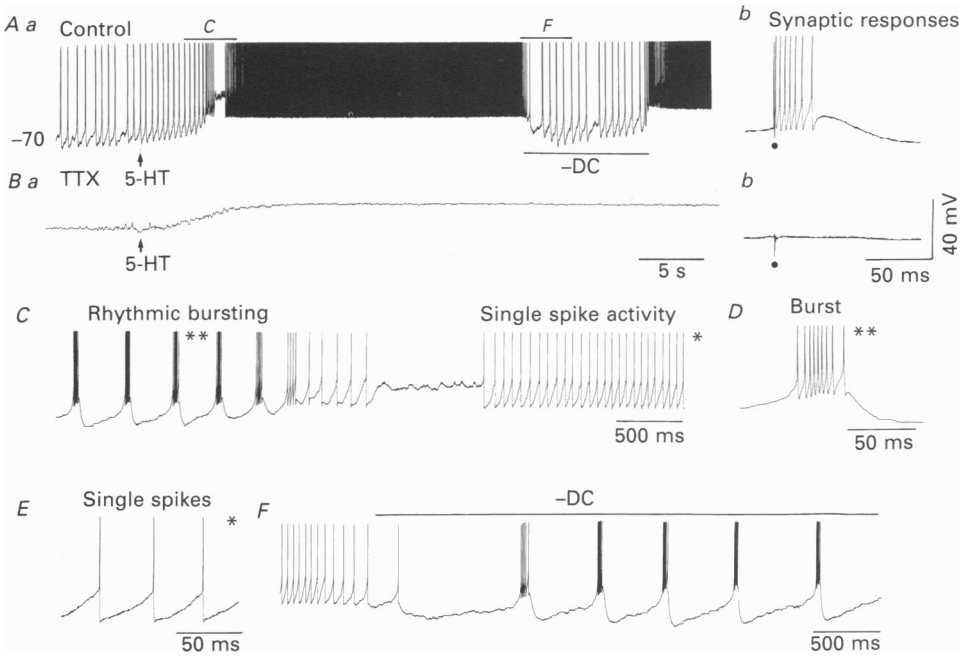

Fig. 5. Serotonin modulates firing mode in NRT neurones. *A*, application of 5-HT to a rhythmically bursting NRT neurone resulted in a depolarization of the membrane potential which was associated with an initial increase in the rate of burst generation followed by a complete inhibition of bursts and the appearance of single-spike activity (transition expended in *C* for detail). Repolarization of the membrane with intracellular injection of current (−DC) reinstated the rhythmic burst firing (expanded in *F* for detail). Local application of tetrodotoxin (TTX, 10 μM) was used to block all stimulus-evoked synaptic transmission (cf. *Ab* and *Bb*). After block of synaptic transmission, application of 5-HT still depolarized this neurone, indicating that this is a direct response. A single burst is expanded for detail in *D*, as indicated, while an example of single-spike activity is expanded in *E*. All data are from the same guinea-pig NRT cell which had an input resistance of approximately 500 MΩ. Action potentials amplitudes are not fully represented.

the burst-firing mode, activation of an EPSP gave rise either to a single burst (Fig. 5*A*, synaptic response), or to a short oscillatory sequence of three to five bursts at a frequency of 4–7 Hz (not shown). Repetitive activation of the EPSPs at different frequencies gave rise to repetitive bursts only at low frequencies of stimulation (< 12 Hz). At frequencies higher than approximately 12 Hz, burst firing failed and the neurone now either became silent or generated a single spike to each EPSP. In contrast, depolarization of NRT neurones into the single-spike firing mode greatly increased the frequency-following capabilities of NRT neurones such that the cells

were then capable of firing to each EPSP at rates greater than 50–100 Hz. This marked difference in frequency-following capabilities appeared to be due to endogenous properties of NRT neurones, for identical results were obtained when short duration (10–20 ms) depolarizing current pulses were used instead of activation of excitatory afferents. These results are essentially the same as those which we have recently reported for thalamocortical relay neurones in the LGND (McCormick & Feeser, 1990).

From these and similar results we conclude that the sequence of neuronal activity which may arise after the application of 5-HT or NA may result entirely from the ensuing depolarization of the membrane potential and from an increase in apparent input resistance (see below). Thus, tonic release of NA and/or 5-HT in the NRT may be expected to result in the promotion of single-spike firing through depolarization of the membrane, while removal of these two neurotransmitters may result in hyperpolarization of NRT neurones, and subsequently the appearance of rhythmic burst firing as the membrane potential passes through the region in which this type of activity is prevalent.

Ionic basis of 5-HT- and NA-induced excitatory responses

Previous investigators have shown that application of 5-HT can result in prolonged excitation by one of three ionic mechanisms: in prepositus hypoglossi neurones application of 5-HT results in depolarization and the generation of action potentials through the enhancement of the hyperpolarization-activated cation current known as I_h (Bobker & Williams, 1989); in nucleus accumbens neurones application of 5-HT results in a slow depolarization and activation of action potentials through a decrease in an inwardly rectifying K^+ current (North & Uchimura, 1989); and finally in human neocortical and rodent hippocampal pyramidal cells, application of 5-HT results in slow depolarization through block of the voltage-dependent K^+ current I_M with a possible contribution of a second, non-voltage-dependent K^+ current (Andrade & Nicoll, 1987; Colino & Halliwell, 1987; McCormick & Williamson, 1989). The possibility that the 5-HT- and NA-induced excitations seen here were mediated by similar ionic mechanisms was examined through bath applications of either Cs^+, which completely blocks I_h, or Ba^{2+}, which has been reported to block I_M and the inwardly rectifying K^+ current of nucleus accumbens neurones (Constanti, Adams & Brown, 1981; North & Uchimura, 1989). Interestingly, prolonged bath application of Cs^+ (4–5 mM; $n = 4$) or Ba^{2+} (400–500 μM; $n = 4$) failed to block, or even reduce, excitatory responses to NA or 5-HT (not shown). These results suggest that the excitatory response examined here is mediated by alteration of an ionic current other than I_h, I_M, or the inwardly rectifying K^+ current detailed in nucleus accumbens neurones (North & Uchimura, 1989). Therefore the characteristics of this current were investigated further.

Application of either 5-HT or NA resulted in a slow depolarization which resulted in the occurrence of single-spike activity ($n = 27$; Fig. 6). Compensation for this slow depolarization with the intracellular injection of current revealed that it is associated with a 1–5 nS decrease in apparent input conductance and lengthening of the membrane time constant (5-HT, $14{\cdot}4 \pm 3{\cdot}6$ (S.D.) ms pre-current injection; $20{\cdot}2 \pm 3{\cdot}2$ ms post-current injection; NA, $14{\cdot}9 \pm 3{\cdot}6$ ms pre-current injection;

$23{\cdot}7 \pm 4{\cdot}5$ ms post-current injection). This result indicates that 5-HT and NA are reducing an ionic conductance in these neurones which is active at resting membrane potentials. In contrast, application of ACh resulted in a pronounced hyperpolarization and increase in apparent input conductance, as previously reported ($n = 7$; McCormick & Prince, 1986).

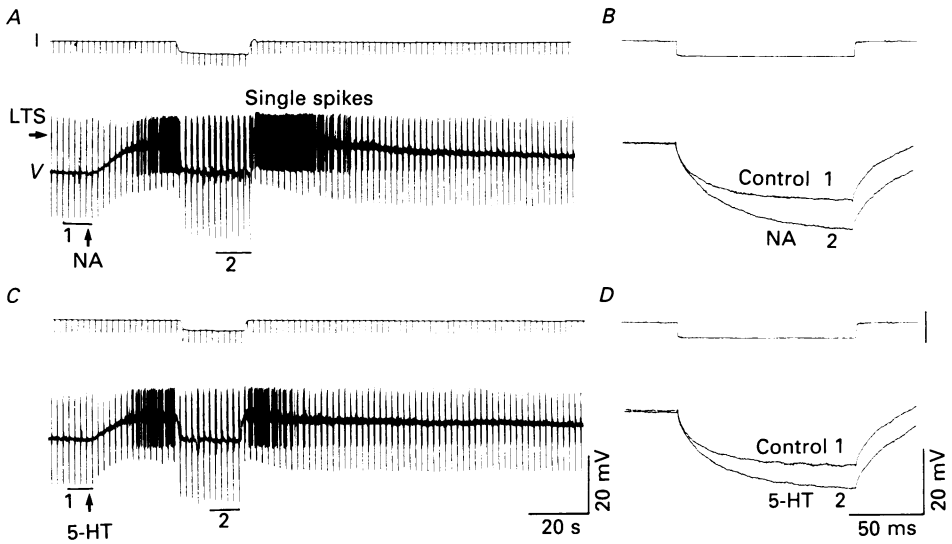

Fig. 6. Noradrenaline- and 5-HT-induced slow excitations result from membrane depolarization associated with a decrease in apparent membrane conductance. *A*, application of NA results in a slow depolarization, a block of rebound low-threshold Ca^{2+} spikes (LTS), and the generation of single-spike activity. Compensation for the slow depolarization with the injection of current (see top trace) reveals the response to be associated with a substantial decrease in apparent membrane conductance. *B*, comparison of the average electrotonic membrane deviations in response to the constant current pulse before (1, control) and during the NA-induced response (2, NA) reveals a substantial decrease in apparent input conductance and a lengthening of the membrane time constant (from 17 to 29 ms). *C* and *D*, application of 5-HT reveals similar results. All data are from the same guinea-pig NRT cell. Action potential amplitudes are truncated for illustrative purposes.

The properties of the current affected by 5-HT and NA were examined by obtaining *I–V* plots with the technique of single-electrode voltage clamp before and during the response to these two neurotransmitters. Both neurotransmitters caused a substantial inward current which reversed polarity at an average of $-107 \pm 4{\cdot}6$ mV for 5-HT ($n = 3$) and -110 ± 7 mV for NA ($n = 5$; Fig. 7; $[K^+]_o = 2{\cdot}5$ mM). Application of ACh, which is known to cause an increase in potassium conductance (McCormick & Prince, 1986), to the same neurones resulted in an outward current which reversed polarity at the same membrane potential ($-110 \pm 5{\cdot}5$ mV; $n = 7$). Subtraction of the control *I–V* plots from those obtained during the transmitter response revealed the relationship between the affected current and membrane potential. The 5-HT- and NA-induced inward currents appeared to be relatively linear in the voltage range -140 to -60 mV (Fig. 7*D*). Similarly, the outward

current activated by ACh was also largely linear in this voltage range (Fig. 7*D*), although in some neurones, the ACh-induced current appeared to display inward rectification at membrane potentials positive to −60 mV (not shown). These results indicate that 5-HT and NA depolarize NRT neurones through a decrease in a

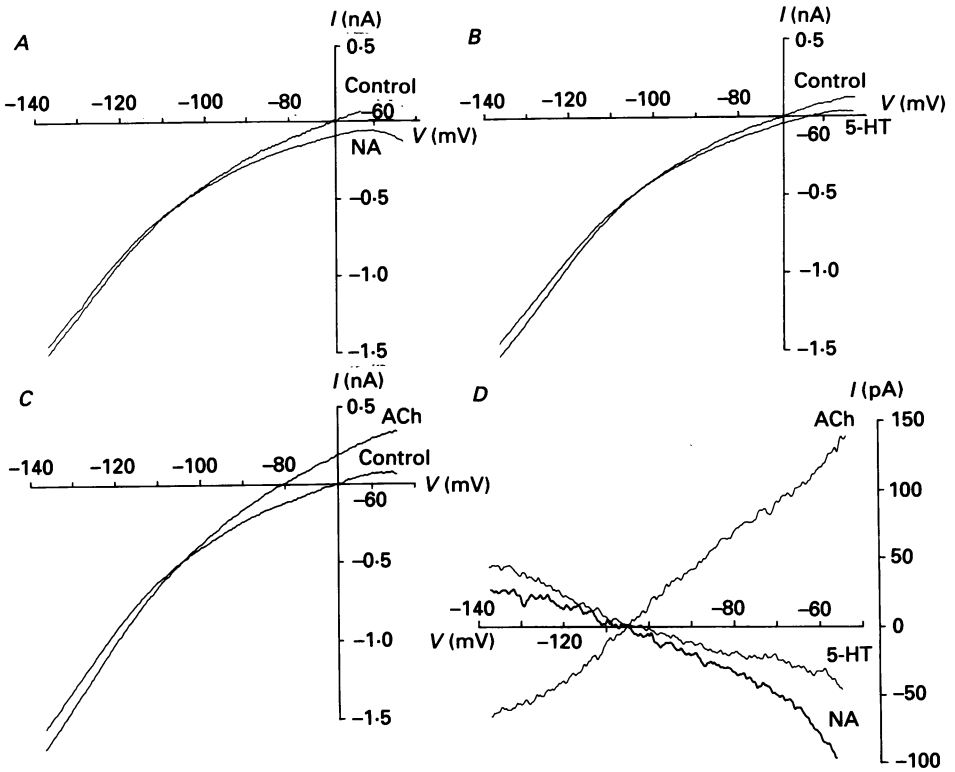

Fig. 7. Voltage characteristics of the ionic responses to NA, 5-HT and ACh. *A*, *I–V* plot before and during the response to NA obtained with single-electrode voltage clamp. NA induces an inward current which reverses to an outward current at −105 mV. *B*, 5-HT also induces an inward current which reverses at −105 mV. *C*, application of ACh results in an outward current which reverses polarity at −105 mV. *D*, subtracting control traces from drug traces reveals the voltage dependence of the currents and their reversal potentials. All three agents reverse at exactly the same membrane potential (−105 mV). All data are from the same guinea-pig NRT cell.

relatively linear potassium current. Here we term this potassium current I_{KL} because of its substantial contribution to the resting 'leak' conductance of the cell, with the understanding that a number of other currents (such as the hyperpolarization activated cation current I_h, McCormick & Pape, 1990) can also contribute to the apparent 'leak' conductance of the membrane and the characteristics of the *I–V* relationship.

The possibility that 5-HT and NA may be decreasing the same potassium current was investigated through the property of non-additivity. In extracellular single-unit recordings, maximal activation of either adrenergic or serotoninergic receptors resulted in a substantial reduction or abolition of the response to the other agonist

($n = 5$). Likewise, in intracellular recordings, the depolarization induced by one neurotransmitter was largely reduced or blocked by maximal activation of the response to the other ($n = 4$; Fig. 8). In contrast, submaximal responses to either agonist gave rise to responses to the other agonist which together added to the

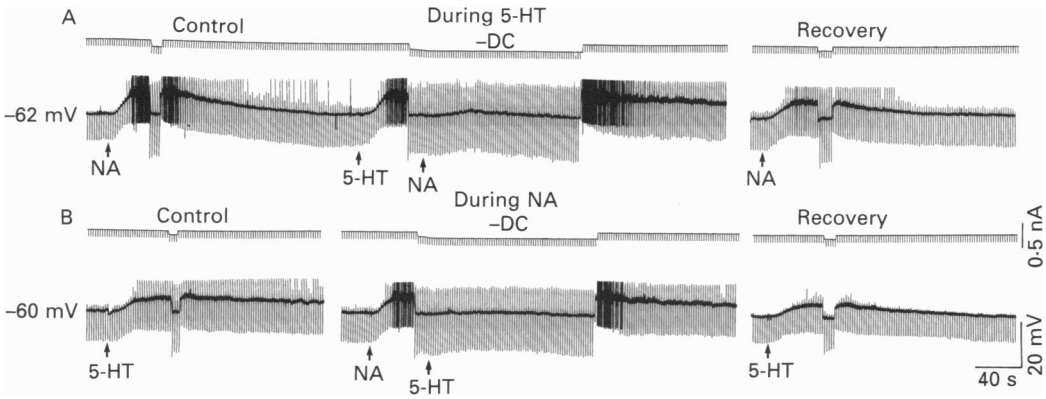

Fig. 8. Noradrenaline- and 5-HT-induced depolarizations are non-additive. *A*, application of NA results in a marked slow depolarization and single-spike activity. Repolarization of the membrane reveals a substantial decrease in apparent input conductance (Control). Application of a large dose of 5-HT to achieve a maximal response also results in depolarization of the membrane. Repolarization with the intracellular injection of current (−DC) reveals a decrease in apparent input conductance. Application of NA at this time results in only a small depolarization. This effect is reversible (Recovery). Similarly, maximal activation of the NA response occludes the response to 5-HT (*B*). All data are from the same guinea-pig NRT cell.

'ceiling' (the response amplitude which was subsequently determined to represent the maximal response). The most parsimonious explanation for this result is that activation of 5-HT_2 (5-HT_{1C}?) and α_1-receptors results in the closure of the same class of potassium channels, although it is also possible that both are competing for a limited supply of the same second messenger.

DISCUSSION

The present results indicate that 5-HT and NA have potent and direct excitatory actions on the GABAergic neurones of the thalamic reticular nucleus. This postsynaptic response appears to result from the suppression of a potassium current (I_{KL}) whose amplitude varies relatively linearly with the difference between membrane potential and the equilibrium potential for K^+ (see Fig. 7*D*). Consistent with this hypothesis, the reversal potentials of the 5-HT- and NA-induced responses were identical to that of the ACh-induced outward current, which we have shown previously to be mediated by K^+ (McCormick & Prince, 1986). The relatively linear nature of I_{KL}, as well as its persistence in high concentrations of extracellular Ba^{2+} and Cs^{2+} and after block of Ca^{2+} currents indicates that it is distinct from the voltage-dependent K^+ current I_M, the Ca^{2+}-activated K^+ current I_{AHP}, and the hyperpolarization-activated cation current I_h, all of which have been shown to be under

the influence of serotoninergic and/or noradrenergic receptors (McCormick & Williamson, 1989; Pape & McCormick, 1989; reviewed by Nicoll, Malenka & Kauer, 1990). The relatively linear characteristic of I_{KL} has the functional consequence that its reduction will result in substantial depolarization at all membrane potentials where NRT neurones may reside *in vivo* (e.g. −80 to −50 mV).

The potent block of the depolarizing response to NA with the α_1-antagonist prazosin, but not the β- or α_2-antagonists propranolol and yohimbine indicates that this response is mediated by the α_1-subtype of adrenoceptors. This hypothesis is supported by the finding that the α-agonist phenylephrine mimicked the actions of NA, while the β-agonist isoprenaline did not and is consistent with the dense presence of α_1-adrenoceptors in the NRT (Jones, Gauger & Davis, 1985). Similar slow depolarizations due to a decrease in potassium conductance upon stimulation of α_1-adrenoceptors have been reported in other regions of the thalamus (McCormick & Prince, 1988; McCormick, 1989), in neurosecretory neurones of the hypothalamus (Bourque, 1988) and in serotoninergic neurones of the dorsal raphe (Aghajanian, 1985).

The receptor mediating the slow depolarizing response to 5-HT appears to be either of the 5-HT_2 or 5-HT_{1C} classes, since this response is blocked by the 5-HT_2/5-HT_{1C} antagonists ketanserin and ritanserin and not blocked by the 5-HT_{1A}/5-HT_{1B} antagonist pindolol or the 5-HT_3 antagonist ICS 205–930. This proposed involvement of the 5-HT_2 or 5-HT_{1C} receptors is also suggested by the mimicry of the 5-HT response by the agonist α-methyl-5-HT, and the failure of obtaining responses to the 5-HT_{1A} agonist 8-OHDPAT or the 5-HT_3 agonist 2-methyl-5-HT (Fig. 3). Although our data at present can not distinguish between 5-HT_2 and 5-HT_{1C} receptors, detailed analysis of a similar depolarizing response in the nucleus accumbens has revealed affinity coefficients for ketanserin which are consistent with mediation by 5-HT_2, and not 5-HT_{1C}, receptors (North & Uchimura, 1989).

Functional consequences of 5-HT and NA actions on NRT neurones

Neurones in the NRT, like most other thalamic neurones, exhibit two distinct patterns of action potential generation: rhythmic burst firing and single-spike activity. Which of these two states these neurones exhibit depends strongly upon the behavioural state of the animal. Rhythmic burst firing is prevalent during periods of inattentiveness, drowsiness, and slow wave sleep, while single spike firing is prevalent during periods of attentiveness and vigilance (see Steriade & Llinás, 1988). The ability of NRT neurones to exhibit these two modes of action potential generation is due to the presence of a strong low-threshold Ca^{2+} current, or T-current (Jahnsen & Llinás, 1984*a*, *b*; Avanzini *et al.* 1989), and the interconnections between these neurones (Steriade, Deschênes, Domich & Mulle, 1985). The low-threshold Ca^{2+} current is inactivated at membrane potentials positive to approximately −60 mV. Thus, in neurones which are tonically depolarized positive to −55 mV, the neurone fires in trains of action potentials in accordance with the bombardment of excitatory and inhibitory synaptic activity from the cerebral cortex, thalamus, brainstem, and other NRT neurones. In contrast, brief (> 50 ms) or prolonged hyperpolarization of the membrane negative to approximately −60 mV removes the inactivation of the T-current, thereby allowing for the generation of low-threshold Ca^{2+} spikes, which

then trigger high-frequency (300–500 Hz) bursts of two to eight fast Na^+- and K^+-dependent action potentials (e.g. see Fig. 5). The interaction of this low-threshold Ca^{2+} spike with other currents, such as a Ca^{2+}-activated K^+ current (Avanzini *et al.* 1989) may allow these cells to exhibit endogenous oscillatory properties (e.g. Figs 4 and 5). Interconnections of NRT neurones with other NRT neurones and with the relay neurones in thalamic nuclei are believed to be critical to the genesis and synchronization of certain thalamocortical rhythms, such as spindle waves (Steriade & Deschênes, 1984; Steriade & Llinás, 1988).

The shift in firing mode among NRT cells, and indeed, almost all thalamic neurones, from rhythmic oscillation to single-spike firing may be determined by the status of activity in ascending serotoninergic, noradrenergic, and cholinergic systems from the brainstem and descending cholinergic projections from the basal forebrain (Steriade & Llinás, 1988; McCormick, 1989). Our present results indicate that increased release of NA and/or 5-HT may result in depolarization of NRT cells out of the rhythmic burst firing into the single-spike mode of action potential generation through a decrease in a relatively linear potassium conductance which contributes substantially to the resting membrane conductance (Fig. 5). Thus activation of these two monoaminergic systems will promote the occurrence of single-spike activity in NRT neurones and therefore will promote a state of activity which has been associated with an awake and attentive animal (Steriade *et al.* 1986). The shift from oscillation to tonic firing of NRT neurones will facilitate the abolition of thalamic oscillations since at least some of these (e.g. spindle waves) depend critically upon burst firing in the NRT (Steriade *et al.* 1985; Steriade, Domich, Oakson & Deschênes, 1987*a*). The increase in tonic, single-spike activity will substantially increase the 'linearity' of NRT neurones in that the pattern of action potentials which they generate will be more representative of the trains of EPSPs which these cells receive from thalamocortical and corticothalamic fibres (e.g. see McCormick & Feeser, 1990). This increase in linearity of NRT responsiveness will facilitate the presence of the waking state in the thalamus as a whole (see McCormick, 1989).

An interesting and functionally important observation in the present study was the occurrence of rhythmic burst firing in some neurones during the ascent, and more often, during the descent of the excitatory response to 5-HT and NA (e.g. Fig. 1). Although we have not yet revealed the exact ionic basis for the appearance of this activity, intracellular recordings suggest that it is due to an interaction of the depolarization and increased apparent input resistance resulting from application of 5-HT and NA and the intrinsic properties of NRT neurones (e.g. Figs 4 and 5). Nucleus reticularis neurones tend to oscillate as individuals *in vitro* only at membrane potentials between approximately -70 and -55 mV (Fig. 4; see also Avanzini *et al.* 1989). Passing the membrane potential through this voltage range will therefore result in increased probability of rhythmic oscillation. The increase in apparent input resistance and lengthening of membrane time constant induced by 5-HT and NA probably can also facilitate the ability of the neurone to oscillate. In this manner, application of 5-HT and NA to an NRT neurone hyperpolarized into the burst firing mode may result in the activation of a low-threshold Ca^{2+} spike which generates a high-frequency burst of fast Na^+- and K^+-mediated action potentials. This burst of action potentials is followed by an after-hyperpolarization which may

be mediated by the activation of a Ca^{2+}-activated K^+ current (Avanzini *et al.* 1989) or the deactivation of the hyperpolarization-activated cation current I_h (McCormick & Pape, 1990). The rising phase of the after-hyperpolarization as well as the continued depolarization of the neurone through the action of 5-HT or NA will then result in another low-threshold Ca^{2+} spike. The rate at which these Ca^{2+} spikes will be generated is determined by the kinetic properties and interactions of the low-threshold Ca^{2+} current and the currents generating the after-hyperpolarization, as well as the strength of depolarization mediated by 5-HT and NA. As the membrane potential becomes depolarized positive to approximately -55 mV, rhythmic burst firing is replaced by tonic single-spike activity, due to inactivation of the low-threshold Ca^{2+} current. These results suggest that although moderate or strong activation of serotoninergic and noradrenergic receptors on NRT neurones is eventually anti-oscillatory and promotes single-spike firing, rhythmic burst firing may occur during the ascent or descent of 5-HT and NA responses (e.g. Figs 1*C*, *D* and *E* and 4). Indeed, extracellular recordings *in vivo* from dorsal raphe and locus coeruleus neurones during various states of sleep and arousal in the cat and rat have demonstrated a clear relationship between the activity of these neurones and the occurrence of spindle waves in the electroencephalogram (Trulson & Jacobs, 1979; Aston-Jones & Bloom, 1981), which are generated through rhythmic burst firing in NRT neurones (Steriade & Deschênes, 1984). From 1–5 s prior to the occurrence of a spindle wave, dorsal raphe and locus coeruleus neurones exhibit a marked inhibition or slowing of firing. Raphe unit activity returns near the end of the spindle wave, while activity of locus coeruleus neurones can actually increase over baseline during the spindle wave. After the spindle wave, the firing rate of both cell groups returns to normal. We suggest that the slow, tonic activity of raphe and locus coeruleus neurones prior to the appearance of spindle waves maintains a tonic depolarization of NRT neurones just above the membrane potentials in which rhythmic oscillation is prevalent. The relaxation of this tonic depolarization by the cessation of neuronal activity in the locus coeruleus and dorsal raphe may then allow NRT neurones to hyperpolarize into the region of oscillatory burst activity, which then expresses itself as a spindle wave, due to the intrinsic properties and interconnections of NRT neurones. A return of firing in raphe and locus coeruleus neurones may then facilitate the cessation of this oscillation by once again depolarizing the neurones out of the membrane potential range for burst firing.

An increase in arousal, or awakening from sleep, on the other hand, is associated with a marked increase in activity in both the dorsal raphe and the locus coeruleus (Trulson & Jacobs, 1979; Aston-Jones & Bloom, 1981). The subsequent increase in release of 5-HT and NA may then depolarize NRT neurones by an amount sufficient to reach (or come near to) single-spike firing threshold, thereby shifting these neurones out of the oscillatory range into the membrane potential range associated with the waking and attentive state. In this manner, serotonergic and noradrenergic inputs from the brainstem may contribute to, and be an important component of, the ascending control of neuronal activity in thalamocortical systems (see also McCormick, 1989).

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