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INTRINSIC PROPERTIES OF NUCLEUS RETICULARIS THALAMI NEURONES OF THE RAT STUDIED *IN VITRO*

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SUMMARY

1. Neurones of the nucleus reticularis thalami of the rat were studied by intracellular recordings from *in vitro* slices. The resting membrane potential was $-56{\cdot}28 \pm 5{\cdot}86$ mV (mean value $\pm$ S.D.); input resistance was $43{\cdot}09 \pm 9{\cdot}74$ MΩ; the time constant τ was $16{\cdot}51 \pm 3{\cdot}99$ ms. At the resting membrane potential tonic firing is present, while at membrane potentials more negative than -60 mV a burst firing mode gradually prevails.

2. Prolonged depolarizing current pulses superimposed on a steady hyperpolarization consistently activated sequences of burst–after-hyperpolarization complexes. The all-or-none burst response consisted of Na^+-mediated, TTX-sensitive fast action potentials superimposed on a low threshold spike (LTS). The burst was followed by a stereotyped after-hyperpolarization lasting 100–120 ms (BAHP), with a maxima -85 mV. The BAHP was blocked by Cd^{2+} and apamine but not by 8-Br cyclic AMP. The early component of BAHP was significantly attenuated by TEA. The oscillatory rhythmic discharges were abolished by agents which blocked the BAHP.

3. The presence of strong after-hyperpolarizing potentials (SAHP and BAHP) in RTN neurones plays a significant role in determining two different functional states, defined as tonic and oscillatory burst firing modes, respectively.

INTRODUCTION

The nucleus reticularis thalami (RTN) is composed of a sheet-like aggregation of GABAergic small neurones (Houser, Vaughn, Barber & Roberts, 1980) which envelopes the dorsolateral and anterior portions of the dorsal thalamus. It receives collaterals from the corticothalamic and thalamocortical fibres and projects exclusively to the dorsal thalamic nuclei (Jones, 1975); moreover, intrareticular axon collaterals of RTN cells provide an intrinsic GABAergic circuit (Yen, Conley, Hendry & Jones, 1985; Spreafico, Frassoni, Battaglia & Schmechel, 1984; De Biasi, Frassoni & Spreafico, 1986).

Several lines of evidence suggest that the RTN is involved in generation of the oscillatory activity responsible for cortical spindles during states of decreased

vigilance (Steriade & Deschênes, 1984; Steriade, Domich & Oakson, 1986; Steriade, Domich, Oakson & Deschênes, 1987).

Recently Mulle, Madariaga & Deschênes (1986), in an *in vivo* study in cats, investigated using intracellular recording the spindle-related rhythmic spike burst activity observed in RTN neurones during barbiturate anaesthesia. The comparison with the intrinsic membrane properties previously reported by Deschênes, Paradis, Roy & Steriade (1984) in *in vivo* cats and by Jahnsen & Llinás (1984*a*, *b*) in *in vitro* guinea-pig thalamic slices led Mulle *et al.* (1986) to outline some peculiarities in the location and relative predominances of conductances, which could support a specific role for the RTN in pacemaking thalamic spindle oscillation.

In the present study the use of an *in vitro* preparation of thalamic slices allowed a further electrophysiological and pharmacological characterization of membrane properties which account for the different firing modes of rat RTN neurones. Ca^{2+}-dependent outward conductances underlying the after-hyperpolarizations which follow single spike tonic firing or burst discharges could be differentiated and related to the ability of RTN neurones to produce rhythmic activities. A preliminary report of some of these data has been published in abstract (Avanzini, de Curtis & Spreafico, 1987).

METHODS

Experiments were carried out on RTN neurones of young adult Sprague–Dawley rats (150–250 g). The animals were decapitated by guillotine under light ether anaesthesia. The brain was quickly removed and immersed in artificial cerebrospinal fluid at a temperature of 4 °C. A block of brain tissue containing the thalamus was dissected from each hemisphere; 400–500 μm thick horizontal thalamic slices were prepared with a vibratome, transferred to the superfusion recording chamber and incubated for at least 1 h before recordings were started. The temperature of the bathing medium was maintained at 36 °C and a humidified gas mixture of 95% O_2– 5% CO_2 was bubbled into the bath during the experiment.

Under a binocular microscope the RTN was recognized as a thin grey band located between the internal capsule and the external medullary lamina; the thalamocortical radiation fibres were clearly visible as white bundles across the RTN.

Intracellular recordings from the portion of the RTN enveloping the ventrobasal nucleus were performed using glass micropipettes (resistance 80–120 mΩ) filled with 4 M-potassium acetate. Extracellular stimuli were applied through monopolar tungsten electrodes placed in the internal capsule and in the dorsal thalamus, close to the recording site, in order to induce a synaptic activation of the neurones. The membrane potentials were recorded with a Neurodata Instrument Corp. (USA) pre-amplifier provided with a Wheatstone bridge circuit utilized to perform intracellular current injections.

Input resistance values were calculated on the initial linear portion of current–voltage curves produced by measuring the membrane voltage changes induced by 80–100 ms hyperpolarizing current pulses applied through the recording microelectrode. The time constant values were calculated by identifying an exponential function derived from the application of a least-squares criterion to the logarithms of the data points extrapolated by the membrane deflection induced by injections of 0·2–0·4 nA current pulses.

The data were recorded on magnetic tape (Racal FM4D) and analysed off-line by means of a PDP 11/34 Digital computer system, together with a Tekronix 5110 digital oscilloscope and a chart recorder (Linseis type 2045). Only data from those neurones producing prolonged (> 10 min), stable recordings and with a membrane potential over −50 mV were accepted for analysis.

The normal bathing solution contained (in mM): NaCl, 124; KCl, 2·5; $CaCl_2$, 2; $MgSO_4$, 2; NaH_2PO_4, 1·25; $NaHCO_3$, 16; dextrose, 10. When saturated with humidified gas, the pH of the solution was 7·4.

When 1 mM-cadmium (Cd^{2+}) was added to the solution, phosphate, sulphate and $CaCl_2$ were omitted and $MgSO_4$ was replaced with $MgCl_2$. During some experiments pharmacological agents

were added to the perfusion fluid without adjusting the tonicity: 1 μM-tetrodotoxin (TTX) (n = 3); 20 mM-tetraethylammonium (TEA) (n = 4); 100 μM-apamine (n = 3); 1 μM-8-bromo adenosine 3′,5′-cyclic monophosphate (8-Br cyclic AMP) (n = 4). All drugs were purchased from Sigma.

RESULTS

The following data are from forty-six neurones from the portion of the RTN related to the ventrobasal nucleus. The mean resting membrane potential was $-56{\cdot}28 \pm 5{\cdot}86$ mV (n = 28). The mean input resistance was $43{\cdot}09 \pm 9{\cdot}74$ MΩ (n = 21). Rectification was observed in the hyperpolarizing direction for values below -85 mV. In resting conditions the membrane time constant (τ_m), calculated in the interval between 10 and 30 ms after the onset of the current pulse, was $16{\cdot}51 \pm 3{\cdot}99$ ms.

No spontaneous activity was recorded from the RTN neurones. Each spike was followed by a short (20–60 ms, mean value: $35{\cdot}28 \pm 13{\cdot}34$ ms, n = 26) and deep after-hyperpolarizing potential (spike after-hyperpolarizing potential, SAHP), which showed a short time-to-peak (mean value: $2{\cdot}33 \pm 0{\cdot}88$ ms, n = 26) and a slower decay. The SAHP persisted during prolonged recordings with KCl-filled microelectrodes and was not blocked by bicuculline. The spike duration was reversibly prolonged by adding TEA to the perfusion fluid; this was due to a slowing down of the spike repolarization, together with blockage of the SAHP (Fig. 1*A*). Addition of 1 mM-Cd^{2+} to the perfusion medium also blocked the SAHP and induced a slowing down of the action potential repolarization; this effect was reversed 40 min after the control solution was restored (Fig. 1*B*).

Long-lasting depolarizing pulses caused repetitive action potential firing (Fig. 1*C*). Each spike was followed by an SAHP.

No burst or Ca^{2+}-like action potentials were observed at membrane polarizations at and above the resting level. The rate of repetitive firing was strictly dependent on the amplitude of the injected current pulse, and showed a slight early accommodation which was manifest during the first 10–20 ms of the pulse. Firing was sustained throughout the duration of current injection (see Fig. 1*C* and *D*). The accommodation did not vary significantly during TEA-added perfusions (Fig. 1*D*).

A post-train after-hyperolarizing potential followed the repetitive discharge evoked by an intracellular depolarizing pulse (Fig. 1*D*). It lasted about 50–80 ms and decayed rapidly with a time constant similar to that of the SAHP. The increased number of action potentials evoked by increasing the depolarizing pulse duration caused a mild prolongation of this potential and no significant increase in its amplitude, which was significantly and reversibly decreased by TEA perfusions (Fig. 1*D*).

When the membrane potential was hyperpolarized below -60 mV, a different firing mode was generated either by orthodromic activation, by depolarizing pulses or at the break of hyperpolarizing pulses: single spike–SAHPs were followed by an all-or-none burst of spikes superimposed on a slow depolarizing potential (Fig. 1*E* and *F*). The bursts lasted about 25–30 ms (mean value: $34{\cdot}35 \pm 9{\cdot}01$ ms) and were composed of four to nine fast action potentials, whose duration and amplitude were similar throughout the duration of the burst. The initial burst firing rate was 200–300 Hz and showed a slight acceleration, which peaked between the third and fifth action

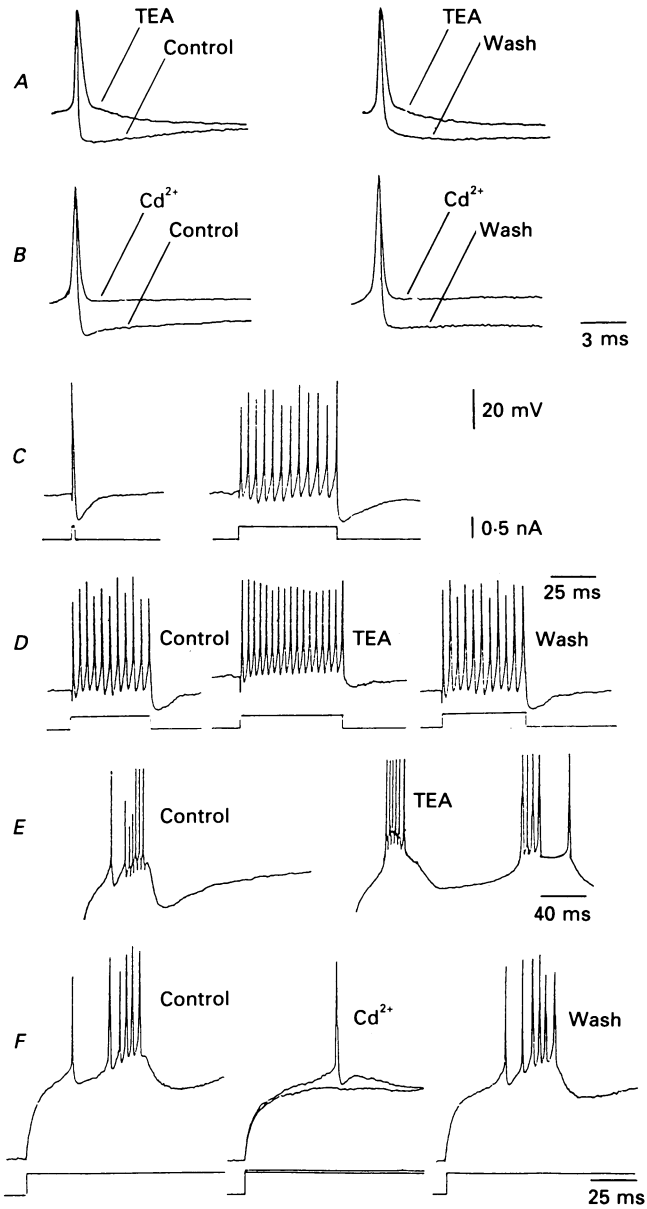

Fig. 1. *A–D*, intracellular recordings from different RTN neurones in resting conditions (single spike–tonic firing mode). *A*, single-spike response evoked by internal capsule stimulation; TEA (10 mM) prolonged the spike duration and reduced the SAHP in a reversible way. *B*, Cd^{2+} (2 mM) has similar effects on the action potential occurring at the break of an hyperpolarizing current pulse. TEA is more effective than Cd^{2+} in prolonging the spike duration. *C*, single-spike response and tonic firing obtained by intracellular injection of depolarizing current pulses of different duration are both followed by short-lasting hyperpolarizations. No important spike accommodation is noted during the repetitive firing. *D*, the after-hyperpolarization subsequent to a train of action potentials is blocked by TEA (10 mM). Note that during TEA addition, the same pulse intensity induced an increase in spike number; this effect is due to the slight depolarization induced

potentials and was followed by a decrease in frequency (Figs 1*F* and 2*B*). On two occasions, during recovery from a low-Ca^{2+}, Cd^{2+}-supplemented perfusion, it was possible to isolate the single spike from the burst response (Fig. 1*F*). When the membrane time constants, obtained by fitting the membrane rectification preceding the single spike and the burst with an exponential function (using a least-squares fitting procedure), were compared a significant difference was detected. The pre-spike time constant of $5{\cdot}6 \pm 1{\cdot}4$ ms was significantly different from the pre-burst time constant of $14{\cdot}5 \pm 0{\cdot}6$ ms ($P < 0{\cdot}05$). The pre-spike rectification was not influenced by Cd^{2+}, indicating that it was no mediated by a Ca^{2+} influx.

The burst was followed by a pronounced hyperpolarization which lasted 80–120 ms and was composed of a fast, TEA-sensitive (Fig. 1*E*) repolarizing phase which peaked at about 20 ms (mean value: $21{\cdot}38 \pm 4{\cdot}9$ ms) and a slower TEA-insensitive (Fig. 1*E*) decay phase (mean value: $96{\cdot}33 \pm 12{\cdot}71$ ms) which decreased monotonically. We have called this after-hyperpolarization a 'burst after-hyperpolarization' (BAHP). The BAHP was an all-or-none phenomenon and showed an approximately extrapolated equilibrium potential of 85–90 mV. The determination of the reversal potential by the injection of hyperpolarizing currents was only tentative, because of the strict dependence of the burst–BAHP complex on the membrane potential. The burst–BAHP complex could not be activated below -90 mV or above -60 mV. It was abolished by Cd^{2+} (2 mM, Fig. 1*F*) while TTX (1 μM) sufficient to abolish Na^+ spikes uncovered a slow all-or-none depolarizing potential underlying the burst (see below and Fig. 3*A*). We have called the all-or-none TTX-resistant Cd^{2+}-sensitive slow potential of the complex 'low threshold spike' LTS (Llinás & Yarom, 1981*a*, *b*; Deschênes *et al.* 1984; Jahnsen & Llinás, 1984*a*, *b*).

Oscillatory behaviour

When RTN neurones are stimulated directly with long-lasting (600–1000 ms) and large (0·3–1 nA) depolarizing current pulses from a hyperpolarized level, repetitive burst–BAHP complexes are elicited, resulting in a characteristic oscillatory behaviour. As shown in Fig. 2*A* and *B* the decay of the outward current responsible for each BAHP results in a depolarizing recovery phase, accompanied by a change in membrane rectification, which leads to a subsequent burst discharge that starts the oscillatory cycle anew. The time constant of the decaying BAHP showed a significant ($P < 0{\cdot}01$) increment just before the activation of the second burst (Fig. 2*B*; see further analysis below). A similar set of events could also be obtained at the break of a large hyperpolarizing pulse injection superimposed on a steady membrane

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by TEA on the membrane potential. *E* and *F*, burst–BAHP complexes elicited in two different RTN neurones by depolarizing current pulses superimposed to a steady hyperpolarizing current which held the membrane potential at 75 and 80 mV, respectively. *E*, the early phase of the BAHP is markedly slowed down during addition of TEA. The isolated action potential preceding the burst in the control recording could not be distinguished from the following burst during the TEA effect. *F*, the isolated potential is not abolished by prolonged Cd^{2+} perfusion, which completely and reversibly blocks the burst discharge. The variability in spike amplitude shown in the fast-speed sweeps in this and the following figures is due to the numeric reconstruction of the events by means of a digital oscilloscope.

hyperpolarization (Fig. 2*A*, right sweep). As shown in Fig. 2*C* the optimal condition to elicit the oscillatory activity was obtained by stepping up the membrane potential from very hyperpolarized levels (below −90 mV in Fig. 2*C* to −85 to −60 mV). Lower depolarizing levels close to the equilibrium potential for BAHP evoked single bursts followed by only a small hyperpolarization, while at higher depolarizing levels close to the resting potential, a first abortive burst was followed by sustained tonic firing (Fig. 2*C*, left sweep).

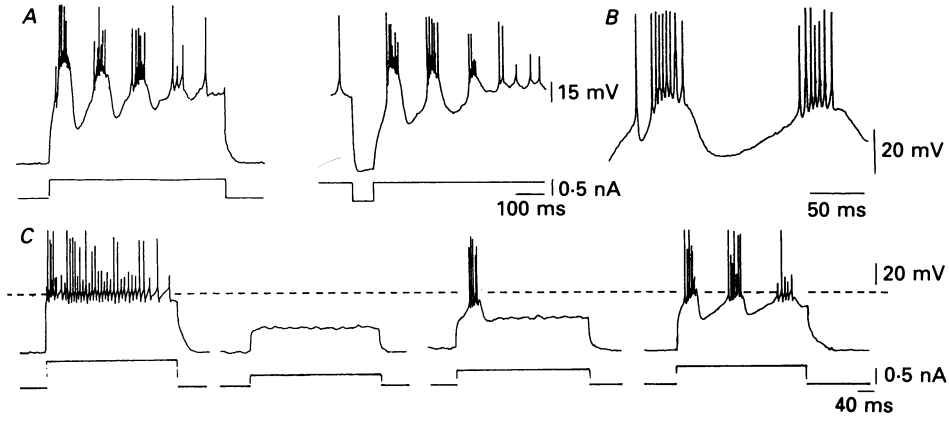

Fig. 2. Oscillatory burst–BAHP sequences in two neurones of the RTN. A, the sequences are activated either by prolonged depolarizing current pulses (left) or at the break of an hyperpolarizing current pulse (right) when the membrane potential is maintained in hyperpolarized conditions by a steady current. *B*, the two early bursts in the left trace in *A* are shown at a greater sweep speed. An isolated action potential precedes the first burst, but not the second and following. *C*, single responses to long-lasting depolarizing pulses starting from a membrane potential kept below −90 mV. Resting potential (dashed line) was −58 mV. When the pulse induced a membrane shift close to resting potential, a tonic response preceded by an abortive burst is activated. At −85 mV the BAHP subsequent to the burst is flattened. Between −85 and −60 mV a deep BAHP is present; its rising phase starts the burst–BAHP sequences anew. The $[K^+]_o$ was 6·25 mM during the recording.

In most of the cells, three to six consecutive oscillations could occur before the oscillatory behaviour faded (Fig. 2*A*). The fade of the sequences was associated with the progressive reduction in the amplitude of the BAHP, presumably due to a gradual reduction in the transmembrane driving force for the ions responsible for the BAHP (Fig. 2*A*). The duration of the burst increased slightly and the initial burst spiking rate slowed down during repetitive sequences (Fig. 2*A*). Note again the single action potential followed by SAHP which precedes by 2–10 ms the first burst activated by a large and abrupt depolarization (Fig. 2*B*).

The addition of TTX to the medium completely abolished the burst Na^+ spikes and isolated the LTS with a rising time constant of 13·34 ± 5·31 ms (Fig. 3*A*) close to the pre-burst time constant values. Note that during TTX perfusion no oscillatory activity could be activated, suggesting that the TTX-sensitive Na^+ component fully expressed in the pre-spike rectification could be relevant in the build up of the regenerative inward currents responsible for the activation of the oscillatory

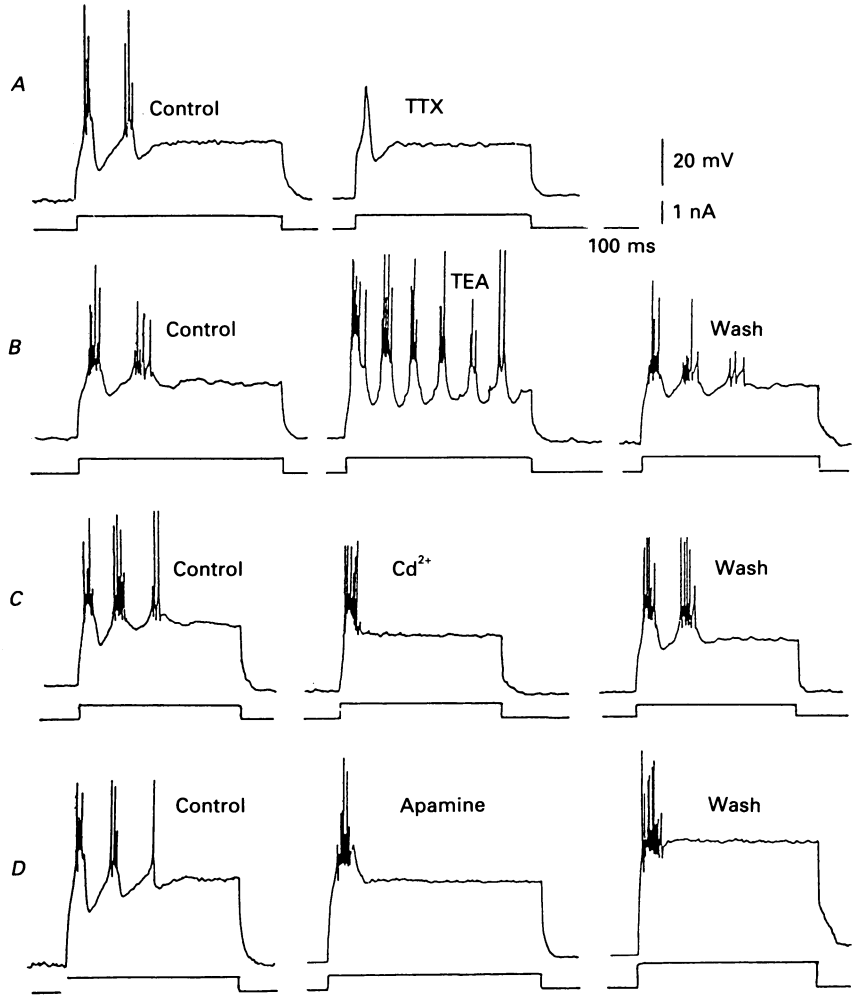

Fig. 3. Effects of different compounds on the oscillatory behaviour induced by depolarizing pulses injected when the cell membrane was maintained at a hyperpolarized level (−90 mV) by a DC current. *A*, when TTX (1 μM) is added to the perfusion fluid, the fast Na^+ spikes forming the burst are blocked, uncovering the LTS. No oscillatory activity follows the activation of LTS when a prolonged depolarizing pulse is delivered. *B*, TEA (10 mM) induces an increase in the oscillating behaviour. The resting potential is −57 mV. *C*, cadmium (Cd^{2+}; 2 mM) added to the medium virtually abolishes the BAHP in an RTN cell. The membrane resting potential is −55 mV. The blockage of the BAHP prevents the burst sequences. The effect of Cd^{2+} was recovered after 40 min of wash-out. *D*, apamine (100 μM) selectively and irreversibly abolishes the BAHP and prolongs the burst duration. No oscillatory repetitive activation is obtained during BAHP blockage, not even when a large depolarizing current pulse is injected (right sweep). Membrane resting potential (−58 mV) is unchanged during apamine perfusion.

behaviour. Figure 3 summarizes the main results of pharmacological tests on repetitive burst–BAHP discharges.

The most noticeable effect of TEA was a considerable enhancement of the tendency to discharge in burst–BAHP rhythmic sequences (Fig. 3*B*), even in those neurones whose oscillating behaviour was less pronounced. The number and the intrinsic rate of the action potentials forming the burst remained virtually unchanged, but the BAHPs showed an increased amplitude and a slower descending phase as compared with control recordings (see above and Fig. 1*E*). During TEA perfusion the single spike which preceded the first burst response during abrupt membrane depolarizations to potentials between -60 and -85 mV could not be separated from the burst itself, due to the abolition of the SAHP.

Exposure to Cd^{2+} first caused a block of the synaptic drive, followed by a slight depolarization of the membrane potential. After a few minutes the BAHP was suppressed (Fig. 3*C*) with a concurrent block of the oscillatory sequences. When the Cd^{2+}-added perfusion was maintained longer, the burst discharges were eventually suppressed, as reported above (Fig. 1*F*).

The 8-Br cyclic AMP, which is known to block selectively the Ca^{2+}-dependent K^+ current, I_{AHP} (Lancaster & Nicoll, 1987), when tested on three cells, had no effect on the BAHP. It also was ineffective on the SAHP and on the post-train hyperpolarization (not shown). Apamine, which is known to selectively and irreversibly block the Ca^{2+}-dependent K^+ currents mediated by the small-conductance Ca^{2+}-activated K^+ (SK-like) ionic channels (Pennefather, Lancaster, Adams & Nicoll, 1985; Blatz & Magleby, 1987) responsible for I_{AHP}, was also tested on three neurones. At a concentration of 100 μM, it strongly reduced the amplitude of the BAHP and prolonged the duration of the burst (Fig. 3*D*). The apamine-induced block of BAHPs was associated with a disappearance of the oscillatory sequences. The SAHP and the hyperpolarization which followed repetitive spike firing in resting membrane conditions was unaffected by apamine (not shown). The effects of apamine were not reversed 1 h after restoring the control solution.

DISCUSSION

Rat RTN neurones studied on an *in vitro* slice preparation show two different firing modes (see also de Curtis, Spreafico, Pauzica & Avanzini, 1988): a single spike tonic discharge at membrane potentials above -60 mV and a burst firing, at membrane levels below -60 mV, responsible for promoting an oscillatory behaviour.

Similar results have been obtained *in vivo* in the rat (Kayama, Sumitomo & Ogawa, 1986) and in the cat (Mulle *et al.* 1986). It has been suggested that these two voltage-dependent responses are related to different functional states of the nucleus. The present study provides new data about the ionic mechanisms underlying the electrical properties which characterize either mode of firing.

The tonic firing pattern is characterized by Na^+ (TTX-sensitive) fast action potentials. Each action potential is followed by a short hyperpolarizing after-potential (SAHP) which is mediated by a Ca^{2+}-dependent K^+ current. This current shows similarities to the I_C, a Ca^{2+}-dependent K^+ current described by Brown & Griffith

(1983) on hippocampal neurones, which is responsible for the spike repolarization and transiently activated by Ca^{2+} influx during spike activation (Brown, Constanti & Adams, 1982). Like I_C (Lancaster & Nicoll, 1987), the current underlying SAHP in RTN neurones has been found to be: (a) K^+ sensitive, (b) blocked by TEA and by Cd^{2+} and (c) reversed at -73 mV (de Curtis *et al.* 1988).

A second TEA-sensitive current is known to be responsible for the spike repolarization, namely the delayed rectifier I_K (Hodgkin & Huxley, 1952). The contribution of I_K to the spike repolarization could be evaluated by subtracting the effects of Cd^{2+} (selectively affecting the Ca^{2+}-dependent I_C) from those of TEA (affecting both the Ca^{2+}-dependent I_C and the I_K) (Fig. 1). As the TEA effect on the repolarizing phase of the action potential was proved to be substantially greater than the Cd^{2+} effect, we concluded that in RTN neurones the I_K significantly contributes to the spike repolarization.

Another Ca^{2+}-dependent K^+ current, the I_{AHP}, is considered to play an important role in spike frequency accommodation on different types of neurones where it underlies long-lasting AHPs (Alger & Nicoll, 1980). In our experiments, the long-lasting SAHPs reported in thalamocortical neurones (Deschênes *et al.* 1984; McCormick & Prince, 1988) were not observed at resting membrane potential in RTN neurones, which accordingly exhibited a poor spike frequency accommodation. Similar findings have been previously reported by Mulle *et al.* (1986) in their *in vivo* study on cat RTN neurones.

The overall result of these data suggests that, in resting conditions, the RTN neurones have strong spike repolarizing currents and that the mechanisms responsible for repetitive firing control are inactive. The short refractory period associated with the lack of accommodation and slow AHP enable the RTN neurones to fire tonically at high frequency. GABAergic neurones, located in different structures of the CNS, have recently been reported to show electrophysiological properties similar to those described above for the RTN neurones, i.e. a fast, high rate of firing with poor frequency adaptation (Schlag & Waszak, 1971; Schwartzkroin & Mathers, 1978; McCormick, Connors, Lighthall & Prince, 1985; Lacaille, Mueller, Kunkel & Schwartzkroin, 1987; Nakanishi, Kita & Kitai, 1987).

The burst response of RTN neurones differs from that of the other thalamic neurones (Jahnsen & Llinás, 1984*a*) in that it is followed by a deep after-hyperpolarization (BAHP). Unlike the AHP described in many CNS neurones, this potential is not activated in a graded fashion and occurs as a transient all-or-none potential. The BAHP was flattened at -85 mV. Its reversal potential was difficult to detect, because of its dependence on the Ca^{2+} influx associated with LTS; when the LTS is inactivated (below -90 mV and above -60 mV) no BAHP was obtained. The BAHP is composed of (a) an early Cd^{2+}- and TEA-sensitive part, probably carried by an outward current which is the result of the summation of the Ca^{2+}-dependent I_C repeatedly activated during the repolarization of the fast action potentials within the burst and (b) a late BAHP due to an outward Ca^{2+}-dependent K^+ current, selectively suppressed by apamine, but not by 8-Br cyclic AMP. Therefore, both the early and the late BAHPs are mediated by Ca^{2+}-dependent K^+ currents, which counteract the strong depolarization underlying the burst. The BAHP plays an important role in the burst termination.

A further difference with respect to the relay thalamic neurones is given by the Na^+ action potential, preceded by a membrane rectification, which leads the burst. This spike is not dependent on the burst, being elicitable when the LTS is blocked by Cd^{2+}; according to Mulle *et al.* (1986), it can result from a persistent somatic or slowly inactivating remote Na^+ current. At membrane polarization levels which enable burst firing, prolonged depolarizing current pulses evoked in RTN neurones sustained, rhythmic sequences of burst–BAHP, which gradually faded. The burst firing frequency varies among neurones, ranging between 6 and 8 Hz. The interburst interval appeared to be directly related to the duration of the BAHP which was never less than 100 ms.

Rhythmic oscillatory activities of 8–12 Hz have been previously reported in intact cat RTN by Steriade & Deschênes (1984), Steriade *et al.* (1986, 1987) and Mulle *et al.* (1986). More recently, McCormick & Prince (1988) observed rhythmic burst discharges at a rate of 0·5–2 Hz in another thalamic nucleus, the paratenial, studied in *in vitro* slices from guinea-pigs.

Our experiments suggest the presence of different currents responsible for bursting sequences in RTN neurones: (a) inward, rapidly inactivating currents carried by Na^+ and Ca^{2+} ions, (b) slow inward regenerative currents and (c) Ca^{2+}-dependent outward currents carried by K^+ ions. A basic role in the maintenance of burst sequences is played by the intracellular Ca^{2+} influx caused by activation of both the LTS and the fast Na^+ spikes which form the burst. When this influx is abolished by Cd^{2+} or TTX, respectively, the burst sequences disappear. The disappearance of rhythmic activity during TTX perfusion could be a consequence of either the abolition of Ca^{2+} influx occurring during Na^+ spike or the blockage of a persistent regenerative Na^+ current (de Curtis *et al.* 1988).

Both the slow rectifying Na^+ current and the Ca^{2+} current responsible for the LTS operated during the depolarizing decaying phase of BAHP. Consistent with the BAHP fading, they became progressively less pronounced and eventually failed to activate a new burst, thereby ending the rhythmic sequence. The Ca^{2+} entry during the burst activates two Ca^{2+}-dependent currents: a fast K^+ current (I_C) and a slow, apamine-sensitive, 8-Br cyclic AMP-insensitive K^+ current. The blockage of the current I_C induced by TEA produces a slowing down of the early BAHP together with an increase in its amplitude and enhancement of the oscillatory behaviour. It was reported recently that TEA could enhance the TEA-resistant Ca^{2+}-dependent K^+ currents, by increasing the Ca^{2+} influx due to the broadening of the spike repolarization (Storm, 1987); it is also known that the TEA-resistant, apamine-sensitive Ca^{2+}-dependent K^+ current is strongly dependent on the intracellular Ca^{2+} concentration (Blatz & Magleby, 1987).

We conclude that BAHP plays a crucial role in deactivating the next LTS in a sequence, thus resulting in an oscillatory activity with a mechanism similar to that recently described by Wilcox, Gutnick & Cristoph, (1988) on the lateral habenula neurones studied *in vitro*. As in the RTN cells, in habenula the burst sequences activated as a rebound response to hyperpolarizing current pulses are dependent on the presence of an AHP which follows the burst. It is noteworthy that in guinea-pig paratenial neurones, where McCormick & Prince (1988) found triphasic AHPs much larger than in RTN, slower oscillatory activities were also found.

On the basis of their intracellular recordings from *in vivo* cat RTN, Mulle *et al.* (1986) suggested that in addition to the K^+-dependent hyperpolarization, GABA-mediated hyperpolarizing IPSPs fulfill an important role in deinactivating LTS in neighbouring neurones. We have found no evidence of any synaptic contribution to the generation of the oscillatory activity in the *in vitro* rat RTN. In addition, local application of GABA to RTN neurones has proved to depolarize rather than to hyperpolarize the membrane (McCormick & Prince, 1986; Spreafico, de Curtis, Frassoni & Avanzini, 1988) and to be associated with a marked decrease in membrane resistance; the resulting shunt of the membrane was usually effective in damping the burst–BAHP sequences.

In conclusion, the reported data are consistent with a double function of isolated RTN neurones studied *in vitro*: (a) a relay-like behaviour, defined by the tonic firing discharge modality and characterized by the ability to perform high frequency responses in a condition of poor repetitive firing control and (b) a bursting discharge behaviour, characterized by the ability to maintain rhythmic burst sequences at low rates (6–8 Hz).

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REFERENCES

ALGER, B. E. & NICOLL, R. A. (1980). Epileptiform burst after hyperpolarization: calcium-dependent potassium potential in hippocampal CA1 pyramidal cells. *Science* **210**, 1122–1124.

AVANZINI, G., DE CURTIS, M. & SPREAFICO, R. (1987). Electrophysiological characterization of single neurones of the nucleus reticularis thalami on rat slices. *Society for Neuroscience Abstracts* **3**, 271.

BLATZ, A. L. & MAGLEBY, K. L. (1987). Calcium-activated potassium channels. *Trends in Neurosciences* **10**, 463–467.

BROWN, D. A., CONSTANTI, A. & ADAMS, P. R. (1982). Calcium-dependence of a component of a transient outward current in bullfrog ganglion cells. *Society for Neuroscience Abstracts* **8**, 252.

BROWN, D. A. & GRIFFITH, W. H. (1983). Persistent slow inward current in voltage-clamped hippocampal neurones of the guinea-pig. *Journal of Physiology* **337**, 303–320.

DE BIASI, S., FRASSONI, C. & SPREAFICO, R. (1986). GABA immunoreactivity in the thalamic reticular nucleus of the rat. A light and electron microscopical study. *Brain Research* **339**, 143–147.

DE CURTIS, M., SPREAFICO, R., PANZICA, F. & AVANZINI, G. (1988). Firing modes of RTN neurons recorded from thalamic slices. In *Cellular Thalamic Mechanisms, Excerpta Medica International Congress Series* 765, ed. BENTIVOGLIO, M. & SPREAFICO, R., pp. 63–75. Amsterdam: Elsevier.

DESCHÊNES, M., PARADIS, M., ROY, J. P. & STERIADE, M. (1984). Electrophysiology of neurons of lateral thalamic nuclei in cat: resting properties and burst discharges. *Journal of Neurophysiology* **51**, 1196–1219.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane currents and its application to conduction and excitation in nerve. *Journal of Physiology* **117**, 500–544.

HOUSER, C. R., VAUGHN, J. E., BARBER, R. P. & ROBERTS, E. (1980). GABA neurons are the major cell type of the nucleus reticularis thalami. *Brain Research* **200**, 341–354.

JAHNSEN, H. & LLINÁS, R. (1984*a*). Electrophysiological properties of guinea-pig thalamic neurones: an *in vitro* study. *Journal of Physiology* **349**, 205–226.

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. (1975). Some aspect of the organization of the thalamic reticular complex. *Journal of Comparative Neurology* **162**, 285–308.

KAYAMA, Y., SUMITOMO, I. & OGAWA, T. (1986). Does the ascending cholinergic projection inhibit

or excite neurons in the rat thalamic reticular nucleus? *Journal of Neurophysiology* **56**, 1310–1320.

LACAILLE, J. C., MUELLER, A. L., KUNKEL, D. D. & SCHWARTZKROIN, P. A. (1987). Local circuit interactions between oriens–alveus interneurons and CA1 pyramidal cells in hippocampal slices: electrophysiology and morphology. *Journal of Neuroscience* **7**, 1979–1993.

LANCASTER, B. & NICOLL, R. A. (1987). Properties of two calcium-activated hyperpolarizations in rat hippocampal neurones. *Journal of Physiology* **389**, 187–203.

LLINÁS, R. & YAROM, Y. (1981*a*). Electrophysiology of mammalian inferior olivary neurones *in vitro*: different types of voltage-dependent ionic conductances. *Journal of Physiology* **315**, 549–567.

LLINÁS, R. & YAROM, Y. (1981*b*). Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurones *in vitro*. *Journal of Physiology* **315**, 569–584.

MCCORMIC, D. A., CONNORS, B. W., LIGHTHALL, J. W. & PRINCE, D. A. (1985). Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons in the neocortex. Journal of Neurophysiology **54**, 782–806.

MCCORMICK, D. A. & PRINCE, D. A. (1986). Acetylcholine induces burst firing in thalamic reticular neurons by activating a potassium conductance. *Nature* **319**, 402–405.

MCCORMIC, 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.

MULLE, C., MADARIAGA, A. & DESCHÊNES, M. (1986). Morphology and electrophysiological properties of reticularis thalami neurons in cat: *in vivo* study of a thalamic pacemaker. *Journal of Neurosciences* **6**, 2134–2145.

NAKANISHI, H., KITA, H. & KITAI, S. T. (1987). Intracellular study of rat substantia nigra pars reticulata neurons in an *in vitro* slice preparation: electrical membrane properties and response characteristics to subthalamic stimulation. *Brain Research* **437**, 45–55.

PENNEFATHER, P., LANCASTER, B., ADAMS, P. R. & NICOLL, R. A. (1985). Two distinct Ca-dependent K currents in bullfrog sympathetic ganglion cells. *Proceedings of the National Academy of Sciences of the USA* **82**, 3040–3044.

SCHLAG, J. & WASZAK, M. (1971). Electrophysiological properties of units in the thalamic reticular complex. *Experimental Neurology* **32**, 79–97.

SCHWARTZKROIN, P. A. & MATHERS, L. H. (1978). Physiological and morphological identification of a non-pyramidal hippocampal cell type. *Brain Research* **157**, 1–10.

SPREAFICO, R., FRASSONI, C., BATTAGLIA, G. & SCHMECHEL, D. E. (1984). The intrinsic organization of the nucleus reticularis thalami in the rat: a Golgi, HRP and immunocytochemical study. *Neuroscience Letters Supplement* **18**, S25.

SPREAFICO, F., DE CURTIS, M., FRASSONI, C. & AVANZINI G. (1988). Electrophysiological characteristics of morphologically identified reticular thalamic neurons from rat slices. *Neuroscience* **27**, 629–638.

STERIADE, M. & DESCHÊNES, M. (1984). The thalamus as a neuronal oscillator. *Brain Research Review* **8**, 1–62.

STERIADE, M., DOMICH, L. & OAKSON, G. (1986). Reticularis thalami neurons revisited: activity changes during shifts in state of vigilance. *Journal of Neuroscience* **6**, 68–81.

STERIADE, M., DOMICH, L., OAKSON, G. & DESCHÊNES, M. (1987). The deafferented reticularis thalami nucleus generates spindle rhythmicity. *Journal of Neurophysiology* **57**, 260–273.

STORM, J. F. (1987). Action potential repolarization and fast after-hyperpolarization in rat hippocampal pyramidal cells. *Journal of Physiology* **385**, 733–759.

WILCOX, K. S., GUTNICK, M. J. & CHRISTOPH, G. R. (1988). Electrophysiological properties of neurons in the lateral habenula nucleus: an *in vitro* study. *Journal of Neurophysiology* **59**, 212–225.

YEN, C. T., CONLEY, M., HENDRY, S. H. C. & JONES, E. G. (1985). The morphology of physiologically identified GABAergic neurons in the somatic sensory part of the thalamic reticular nucleus in the cat. *Journal of Neuroscience* **5**, 2254–2268.