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RESEARCH ARTICLE

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5-Hydroxytryptamine modifies neuronal responses to glutamate in the red nucleus of the rat

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Abstract The effects of 5-hydroxytryptamine (5-HT) on the responses of red nucleus (RN) neurones to glutamate (glu) and its agonists were studied using a microiontophoretic technique in anaesthetised rats. Extracellular unitary recordings of RN neuronal activity showed that 5-HT application induced a significant and reversible depression of glu-evoked excitations in 85% of the RN units tested. This effect was independent of the action of the amine on background firing, which appeared enhanced in the majority of cases but was either depressed or uninfluenced in other cases. Microiontophoretic 5-HT also depressed the excitatory responses evoked in RN neurones by electrical stimulation of sensorimotor cortex. Methysergide application, which prevented the enhancing effects of 5-HT on the background firing, was scarcely effective in antagonising the depression of glu responses. In contrast, the serotonergic effects on the glu responses were reduced by the iontophoretically applied antagonist of 5-HT_{1A} receptors, NAN-190. Microiontophoretic 5-HT was also able to influence the neuronal responses evoked by glu agonists quisqualate (quis) and *N*-methyl-D-aspartate (NMDA), acting on non-NMDA and NMDA receptors respectively. In fact 5-HT depressed quis-evoked excitations and induced mixed effects on NMDA responses, which were reduced in 45%, enhanced in 34% and unmodified in 21% of the units tested. These results suggest that 5-HT is able to modulate the motor glutamatergic input to RN by acting mostly on non-NMDA receptors. The modulation of non-NMDA and NMDA receptors by 5-HT in the RN appears significant and its functional meaning is discussed.

Key words Red nucleus · 5-Hydroxytryptamine · Glutamate · glu receptors · Microiontophoresis

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Introduction

The red nucleus (RN) is a brainstem structure that plays a role in motor control by delivering its output to the spinal cord through the rubrospinal tract and relaying it to the cerebellum by the rubro-olivary path (Massion 1967; Martin and Ghez 1988). The nucleus receives a prominent motor input from cortex and cerebellum, mostly conveyed by fibres using excitatory amino acids as neurotransmitters (Stone 1979; Davies et al. 1986; Bernays et al. 1988; Nieoullon et al. 1988).

A dense innervation by serotonergic fibres and terminals has been also described in the RN of both the cat (Bosler et al. 1983) and the rodent (Steinbusch 1984), the bulk of this input to the RN being provided by the dorsal raphe nucleus (Pierce et al. 1976; Bernays et al. 1988). The presence of serotonergic afferents to the RN is substantiated by high levels of 5-hydroxytryptamine (5-HT) (Palkovitz et al. 1974) and by intermediate numbers of 5-HT receptors (Pazos and Palacios 1985; Pazos et al. 1985), mostly concentrated in the caudal-dorsal zone of the nucleus.

The projections of 5-HT fibres have been classically implicated in the control of pain (Basbaum and Fields 1984), depression (Deakin 1988; Meltzer and Lowy 1987; Kahn et al. 1988) and the sleep-waking cycle (Jouvet 1969; Hobson et al. 1986), but the serotonergic innervation of RN indicates that the influence of 5-HT systems might extend beyond these fields.

A hypothesis advanced by Jacobs and Fornal (1993) is that the primary role of the 5-HT system might be to modulate motor activity. In this view, serotonergic innervation of the RN is a possible substrate for the actions of 5-HT. However, the interaction of 5-HT with other neurotransmitters in the RN has received little attention. Directly applied 5-HT modifies the background firing rate of RN neurones in vivo in cats (Davis and Vaughan 1969) and rats (Licata et al. 1995) and depresses their excitatory response to stimulation of the brachium conjunctivum in the baboon (Huffman and Davis 1977). Given the motor role exerted by the RN and the glutamatergic projection to the

nucleus, a specific interaction between 5-HT and glutamate (glu) could have functional implications in light of the hypothesis outlined above.

In the present study, we tested the effects of microiontophoretic 5-HT on the glu-induced enhancements of firing rate in RN neurones. A secondary goal was to establish whether a possible interaction between 5-HT and glu involved both *N*-methyl-D-aspartate (NMDA) and quisqualate (quis) ionotrophic receptors for the excitatory amino acids.

Materials and methods

The experiments were performed on male Wistar rats, anaesthetised by intraperitoneal injection of urethane (1.5 g/kg). The principles followed for the care and use of laboratory animals conformed to the current version of the Italian law on the protection of Animals and to NIH publication no. 85-23. The temperature of the animal was maintained with a heating pad and the heart rate was continuously monitored during the experiment. Exposed tissue was covered with a gel of Agar-Agar (2%), applied to prevent desiccation. Supplementary doses of anaesthetic were administered whenever the heart rate exceeded the normal rate.

Small holes were drilled in the skull at the coordinates corresponding to the RN (Paxinos and Watson 1986). Five-barrel micropipettes were used for recording extracellular impulse activity from single neurones and simultaneously applying pharmacological agents.

The recording barrel (resistance 7–12 MΩ) was filled with a 4% solution of Pontamine Sky Blue in 3 M NaCl. Action potentials, recorded extracellularly from single RN neurones, were identified on line as neuronal activity and used for data only if the spike amplitude was at least 4 times greater than the noise level and remained unmodified during the tests. Recorded activity was filtered (300 Hz to 10 kHz band-pass signal), amplified, discriminated and fed to a personal computer via Cambridge Electronic design 1401 interface and software, for storage and analysis on and off line.

Prior to recording, each of three barrels of the micropipette was filled with one of the following solutions: 5-HT (Sigma, 30 mM, pH 4.5), monosodium glutamate (glu, Sigma, 100 mM, pH 8), *N*-methyl-D-aspartate (NMDA, Sigma, 100 mM, pH 8), sodium quisqualate (quis, Sigma, 100 mM, pH 8), methysergide bimaleate (mts, Sandoz, 10 mM, pH 4.5–5), 1-(2-methoxyphenyl)-4-(4-phthalimidobutyl)piperazine hydrobromide (NAN-190, Tocris Cookson, 10 mM, pH 4.5–5). All the drugs were made up in distilled water with the exception of 5-HT and NAN-190, which were dissolved in 165 mM NaCl and in diluted dimethyl sulphoxide (DMSO) respectively. If necessary, the pH was adjusted with HCl or NaOH (0.1 M). A barrel filled with 3 M NaCl enabled the automatic current-balancing circuit to neutralise any voltage shift. Retaining currents of 5–10 nA (positive for glu, NMDA and quis, negative for 5-HT, mts and NAN-190) were applied to the barrels to reduce drug leakage during electrode penetrations.

Glu, NMDA and quis were applied with negative current pulses having a duration of 30 s and intensity up to 60 nA. 5-HT and its antagonists were ejected with long-lasting (4–20 min) positive currents (2–40 nA). By passing current up to high intensity (60 nA) through a barrel containing DMSO, it was preliminarily verified during a penetration that DMSO had no effect on the neuronal firing rate.

The number of spikes was calculated and integrated over 1–5 s for display and analysis. Spontaneous activity recorded for at least 3 min before drug tests was used to calculate the mean firing rate [and both the standard deviation (SD) and the standard error (SE)]. This value was defined as the mean background activity and used for statistical analysis. If the SD exceeded 20% of this value, the unit was excluded from further analyses. Whenever a unit slowly modified its mean background activity during the recording session, a new value was calculated.

Routinely, whenever a single neurone was isolated, three microiontophoretic applications (30-s pulses) of glu alone (or an agonist) were followed by three applications performed during continuous 5-HT application. Then, glu (or agonist) was pulsed for at least 5–10 min after cessation of the 5-HT ejection to ascertain recovery. In some cases this sequence of trials (indicated as *complete set* below) was repeated, 5-HT antagonists being applied simultaneously with 5-HT. The cycle of retention-ejection was fixed for each neurone on the basis of the duration of the responses. As a rule, following each trial we waited for the firing rate to return to the initial value and, after a further delay (1–2 min according to the variability of the background) ejected the drug again. Whenever co-ejection of another drug (e.g. 5-HT) or spontaneous oscillations modified the background firing, the application of glu (or an agonist) was delayed.

Following a drug ejection, a *response* was defined as a change in the mean firing rate by at least 2 SD from the mean background activity and lasting more than 20 s. Three parameters were used to describe the response intensity: the magnitude M, the contrast C and the duration D. M was defined as the difference between the numbers of spikes recorded during the response and during a similar period of time preceding drug ejection. The ratio between these two values was defined as the contrast C and was estimated as a signal-to-noise value. Finally D indicated how long the response lasted, i.e. the mean firing rate modification above or below 2 SD from the background.

The effect of 5-HT on the responses to glu (or NMDA or quis) in each unit was expressed in terms of modifications (absolute values and per cent) of the M, C and D values elicited by glu (or NMDA or quis) applied to the same neurone alone. Two-tailed Student's *t*-test (or Mann-Whitney *U*-test for normalised data) was used to compare different sets of single trials recorded under different conditions (before, during and after application of 5-HT, mts, etc.). Paired tests (*t*-test and Wilcoxon rank test) were used to compare the mean values of responses recorded under different conditions in a neuronal population. All the results referred to a population are reported below as mean values±the standard error of the mean (SEM).

The last recording site of each penetration was marked by iontophoretic application of Pontamine Sky Blue (cathodal current: 10–20 μA, 10–15 min).

At the end of the experiment the rat was killed by an overdose of anaesthetic, and the brain removed and kept for 3 days in 10% formalin. The electrode tracks and the recording sites were identified in serial brainstem coronal sections (50 μm thick), stained with Neutral Red.

Cortex stimulation experiments

During three experimental sessions the firing of RN neurones was recorded upon electrical stimulation of ipsilateral sensorimotor cortex before, during and after microiontophoretic application of 5-HT into RN by a barrel of the same recording microelectrode. A nickel-chromium stimulating electrode delivered trains of two or three pulses (0.1–0.2 mA, 0.25 ms, 500 Hz) at a frequency of 0.5 Hz. Post-stimulus time histograms (PSTHs) were calculated, averaging 60–80 trials of the 100–200 ms period following each stimulus train. PSTHs recorded under different conditions were compared using a paired *t*-test.

Results

The neuronal activities of 82 units were recorded, 66 of which were histologically identified as belonging to the RN. Of these, 52 were tested during at least a complete set of trials and 14 were used in control experiments. The background discharge rate of the sample ranged between 5 and 45 spikes/s with a mean value of

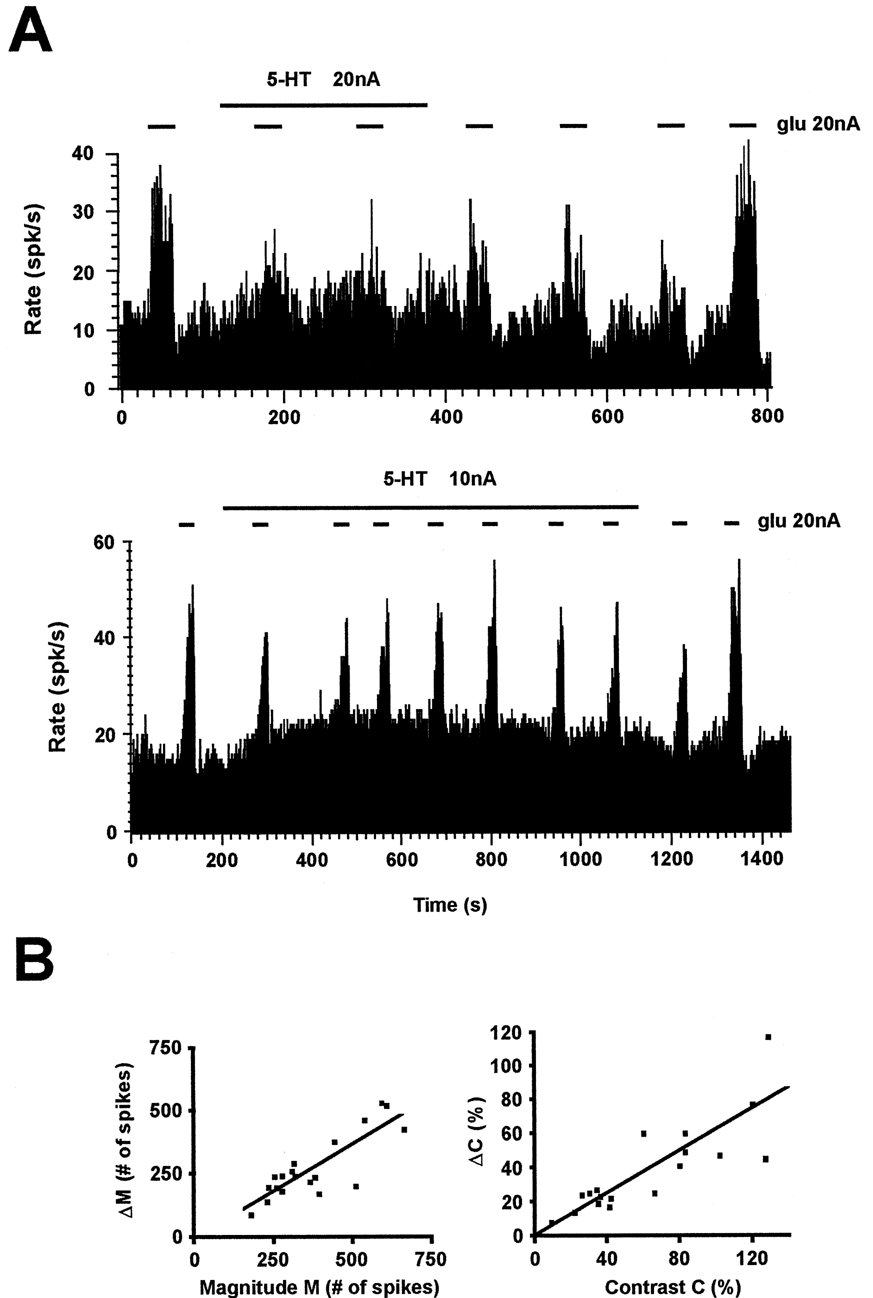

Fig. 1 **A** Rate histograms (1-s bins) showing the inhibitory effects of 5-hydroxytryptamine (5-HT) on the excitatory responses to glutamate (glu) in two neurones of the red nucleus (RN). The *horizontal bars* above the histograms indicate the duration of the ejection periods at the currents given. Responses to glu ejection during iontophoretic application of 5-HT appear attenuated in both traces. Note in the upper trace the slow recovery after 5-HT application and in the lower trace the 5-HT-induced enhancement of the basal firing rate. In the latter, glu responses are attenuated, but not abolished even after 15 min of 5-HT ejection.
B Graphs showing the modifications induced by 5-HT on the magnitude M and the contrast C describing the glu responses. The mean depression of the glu responses by 5-HT (ΔM and ΔC) is reported (whenever significant) as a function of the mean M and C values of the glu response recorded in the same cell in the absence of 5-HT. A significant linear correlation is found in both cases (ΔM vs M: r=0.82, P<0.0001; ΔC vs C: r=0.95, P<0.0001; paired two-tailed t-tests)

Table 1 5-Hydroxytryptamine (5-HT) effects on responses to glutamate agonists. All observations in each column were made on the population of neurones indicated in parentheses. M values are given in number of spikes (±SE), C values in per cent variation (%) (*N* number of significant effects, *Crtl* control values)

		glu (N=22)	quis (N=14)	NMDA (N=29)	
	N	19	8	13	10
M	*Ctrl*	395±40	373±86	435±71	443±62
	+5-HT	166±6	213±73	248±58	647±93
	P	***	***	*	**
	N	18	8	11	9
C	*Ctrl*	78±18	79±23	53±7	35±7
	+5-HT	29±8	40±12	32±6	79±34
	P	***	**	***	**

* P<0.05, ** P<0.01, *** P<0.001

22.01±12.43 spikes/s, a median rate of 20 and a mode around 10–15 spikes/s. These values were not significantly different from those reported in other papers (Billard et al. 1991) with regard to the active units. The high number of silent neurones detected by other authors in the nucleus was probably due to the different type of anaesthesia they used. At least in some cases, however, leakage of glu, despite the retention current, cannot be excluded.

The application of 5-HT consistently depressed the responses to iontophoretic glu in 19 of the 22 neurones tested (Table 1). In fact 5-HT ejection at a low current intensity (2–20 nA) induced a significant decrease in both M and C. This effect was significant in each of these units with regard to M (t-test, P<0.0001) and in 18 of them with regard to C (Mann-Whitney U-test, P<0.0001) val-

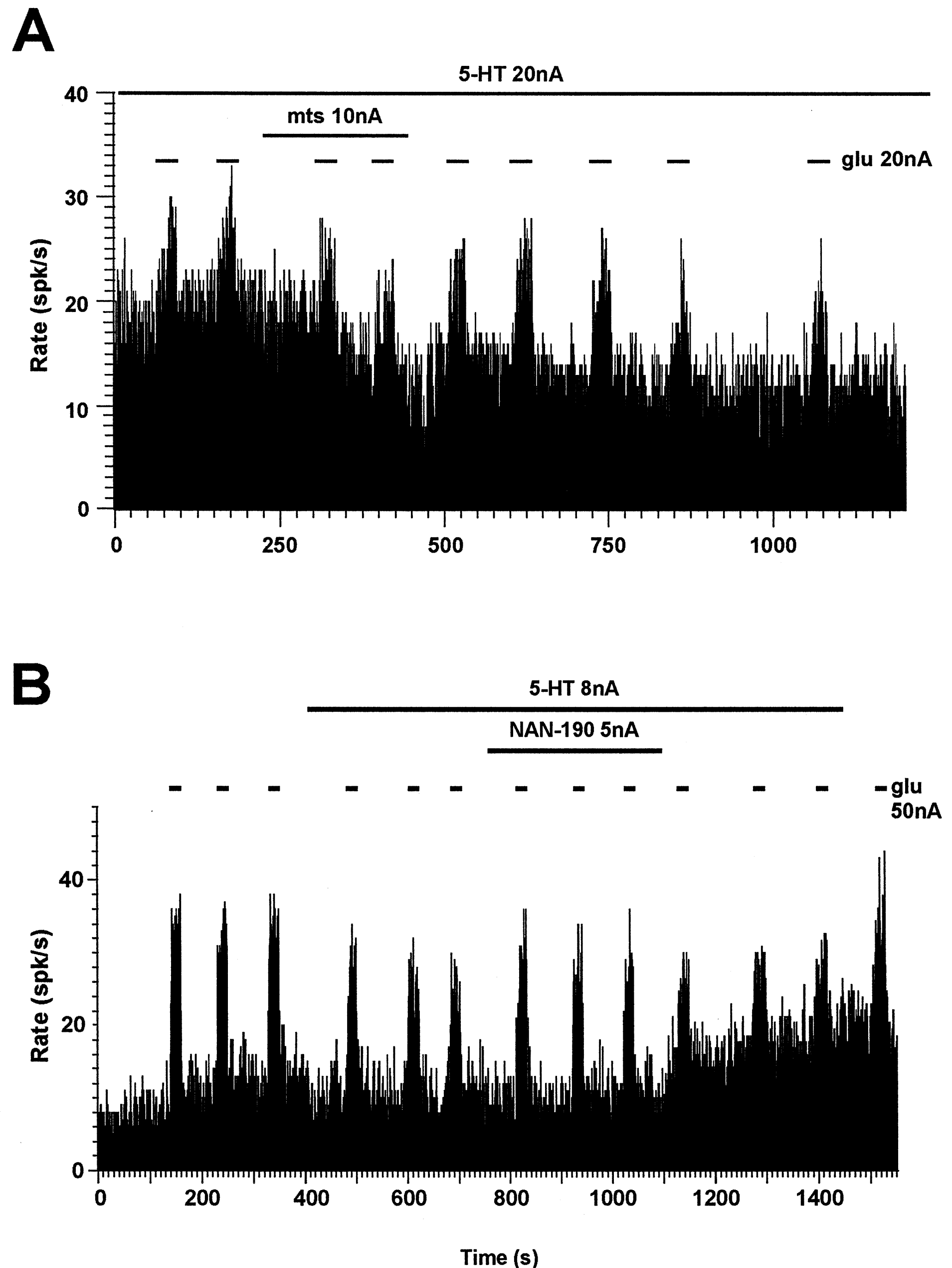

Fig. 2 **A** Rate histograms (1-s bins) showing the effects of the 5-HT antagonist methysergide (mts) on the glu responses of a RN neurone. Mts antagonised the 5-HT-evoked enhancement of the background firing rate whereas it slightly attenuated glu responses, in this regard mimicking the action of 5-HT. **B** Rate histograms (1-s bins) showing the effects of the 5-HT_{1A} antagonist NAN-190 on the glu responses of a RN neurone. 5-HT-induced attenuation of excitatory glu responses was partially antagonised by simultaneous NAN-190 application

ue. On the whole 5-HT was able to reduce glu-evoked excitations to about 40% of their initial magnitude.

Glu responses generally appeared depressed within 1–2 min from the beginning of 5-HT application (Fig. 1A) and recovered 3–15 min from the end of the ejection. The only exception was a unit that exhibited an enhancement of the response followed by a late depression after 2–3 min. In contrast with its effects on the intensity of glu-evoked responses, 5-HT ejection had no effect on their time course, D, in the great majority of cases. In fact a significant reduction was detected only in two units.

Whenever possible, 5-HT was applied at doses which did not modify the background firing. Attenuation by 5-HT of glu-evoked activity was, however, independent of its effects on background firing. In fact a depression of glu responses was observed in RN neurones showing no or small changes of basal activity during 5-HT as well as in those modifying their resting discharge.

The depressive action of 5-HT on the glu responses was dose-dependent, but in the majority of cases unable to abolish the glu-evoked effect totally. In fact, only in six of the 19 responsive units could glu responses be reduced by more than 80%, even when the 5-HT ejection current was enhanced to more than 40 nA.

To test the hypothesis of a direct antagonistic action of 5-HT on glu receptors we analysed the maximal depressions (ΔM and ΔC) of the glu responses induced by 5-HT in each responsive unit, reporting them as a function of the M and C values detected in the same cell before 5-HT ejection (Fig. 1B). The two sets of parameters were

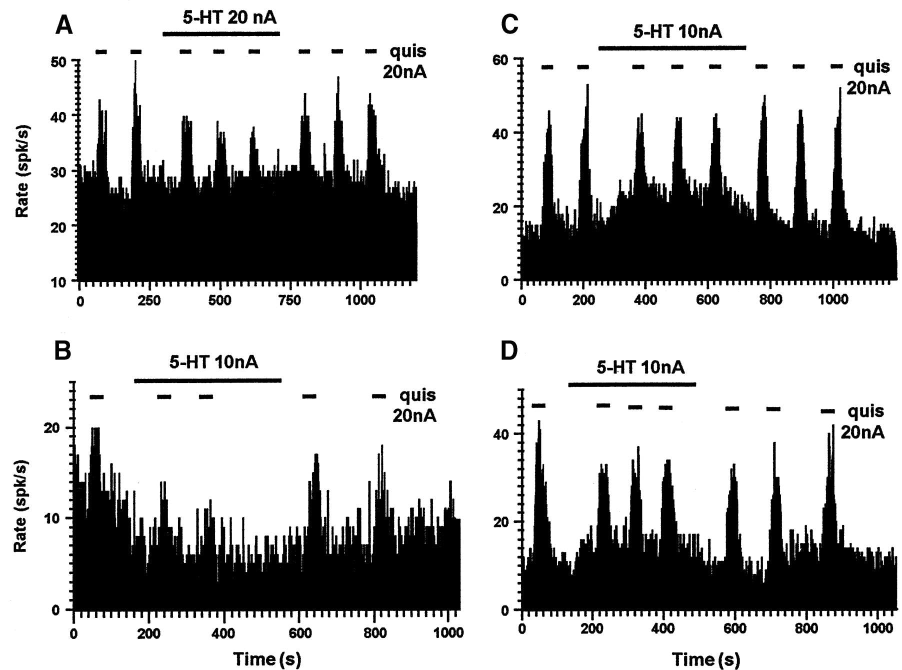

Fig. 3A–D Rate histograms (1-s bins) showing the depressive effects of 5-HT on responses to quisqualate (quis) application in RN neurones. 5-HT attenuated responses to quis application independently of its effects on the background firing, which were either unaffected (**A**), depressed (**B**) or enhanced (**C**, **D**). Note that, after 5-HT ejection, recovery times of the glu responses and basal firing rate were unrelated. In fact a fast recovery of both is recorded in **C**, and a slow recovery of the glu responses in **D**

linearly and positively related (ΔM vs M: r=0.82, P<0.0001; ΔC vs C: r=0.95, P<0.0001, paired two-tailed t-test). Thus, 5-HT ejection was able to reduce large glu responses more than small ones.

Microiontophoretic application of the 5-HT antagonist mts scarcely prevented attenuation of the glu responses by 5-HT. In fact mts, tested in six RN neurones, was ineffective on 5-HT-induced depression of glu responses in three of them, enhanced it in two (Fig. 2A) and antagonised it partially in one case. In contrast, mts antagonised the 5-HT-evoked enhancement of the background firing in five of six neurones and in two cases turned the excitatory response to 5-HT application into a biphasic one, unmasking an inhibitory component.

The 5-HT_{1A} receptor antagonist NAN-190, tested in six RN neurones, was able to reduce the 5-HT effect on glu responses in four of them (Fig. 2B) and was ineffective on glu responses in the remaining two neurones, both unresponsive to 5-HT.

Sporadic enhancements of glu responses by 5-HT were recorded in two of the three units whose background firing was inhibited by 5-HT.

The effects of 5-HT on the activation by microiontophoresis of glu agonists quis and NMDA were also analysed in RN neurones. Both quis- and NMDA-evoked excitatory responses were modulated by 5-HT but its effectiveness was different in the two cases.

Quis, tested on 14 RN neurones, induced excitatory effects similar in shape and duration to glu responses. Quis-evoked excitations were significantly depressed by 5-HT application in eight units (Fig. 3), unaffected in four and enhanced in two.

The depression of quis-evoked responses during 5-HT application (Table 1) affected both M and C, which were reduced to about 57% and 51% of control values respectively. In four RN units the time course D of the responses was also significantly shortened by about 34% (from 42±5 s to 28±3 s, N=4) during 5-HT application. The depressive action of 5-HT on the quis-evoked responses was independent of its effects on background firing (Fig. 3B, C).

The percentage of quis responses depressed by 5-HT and the mean reduction of M and C values suggest that 5-HT is less effective on quis than on glu responses. Differences between the two cases, however, were not statistically significant.

Responses to NMDA ejection during 5-HT application were studied in 29 RN units. NMDA application evoked longer-lasting excitations than glu, a fast enhancement of the firing rate being followed by a slow recovery. During 5-HT application these responses were depressed in 13 units, enhanced in ten and unaffected in six (Table 1). There was no relation between the influence of 5-HT on the NMDA-evoked responses and on the background activity.

In 13 RN neurones, 5-HT depressed the NMDA responses (Fig. 4A, B) reducing M, C and in almost a half the cases also D (from 49±5 to 32±4 s, N=6). On the whole 5-HT was able to reduce NMDA responses to about 60% of their initial value.

In ten RN neurones 5-HT enhanced NMDA-evoked responses (Fig. 4C, D), increasing M, C and in 2 cases also D. The enhancement of NMDA-evoked responses was transient (3–6 min) whatever the duration of 5-HT ejection (Fig. 4D).

The units whose NMDA responses were enhanced during 5-HT ejection were more numerous in the caudal sectors (stereotaxic planes A: 2.70–2.96, see Paxinos and Watson 1986) than in the rostral sectors (A: 3.20–3.40) of the nucleus. The inverse was true for the neurones

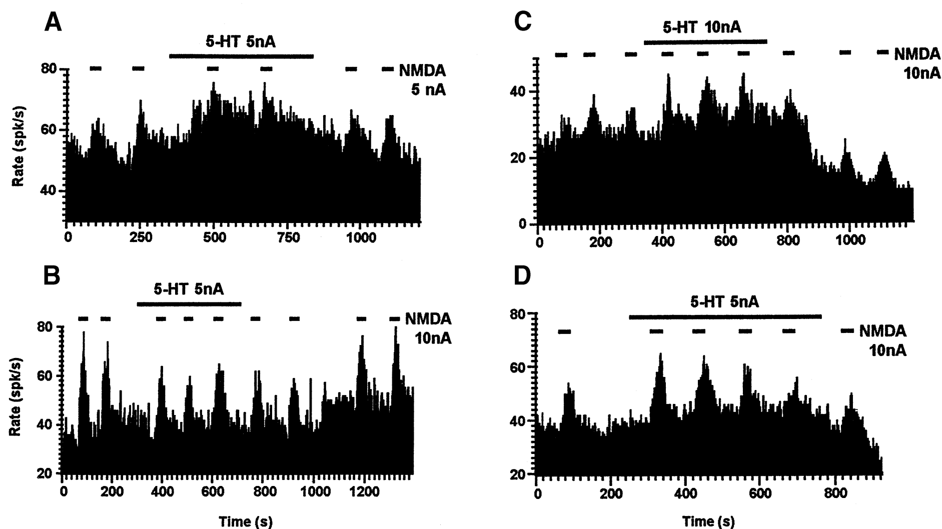

Fig. 4A–D Rate histograms (1-s bins) showing the effects of 5-HT application on the excitatory response to *N*-methyl-D-aspartate (NMDA) in four RN neurones. **A** NMDA responses were depressed and the background firing was enhanced. **B** Both NMDA responses and basic firing rate were depressed. **C, D** Both NMDA responses and background firing rate were enhanced. Note that in **D** the enhancing action of 5-HT on the NMDA responses lasted less than 5 min, although the 5-HT application lasted about 9 min

whose NMDA responses were depressed in the presence of 5-HT. The difference between the two distributions was significant (Fisher's exact test for 2×2 tables, $P<0.05$).

We compared directly the effects of 5-HT on quis and NMDA responses in 12 neurones. In six of them 5-HT had the same depressive action on both responses and in 1 case was ineffective on both. In the remaining five neurones the effect of 5-HT on the two responses was different. In fact, whenever NMDA responses were enhanced by 5-HT (3 cases), quis responses appeared depressed or uninfluenced and, on the contrary, when NMDA responses were uninfluenced (2 cases) quis responses appeared depressed.

On the whole, glu- and quis-evoked responses were similar in shape and duration and the depressive action of 5-HT was also similar on both. NMDA effects differed significantly from the glu effects with regard to C and D values and were variously modulated by 5-HT, either depressive or enhancing effects being observed in a significant group of cells.

Cortex stimulation experiments

Low-frequency trains of two or three pulses (see Materials and methods) were applied to the sensorimotor cortex during the recording of neuronal activities of 14 neurones in the course of three experimental sessions. The PSTHs,

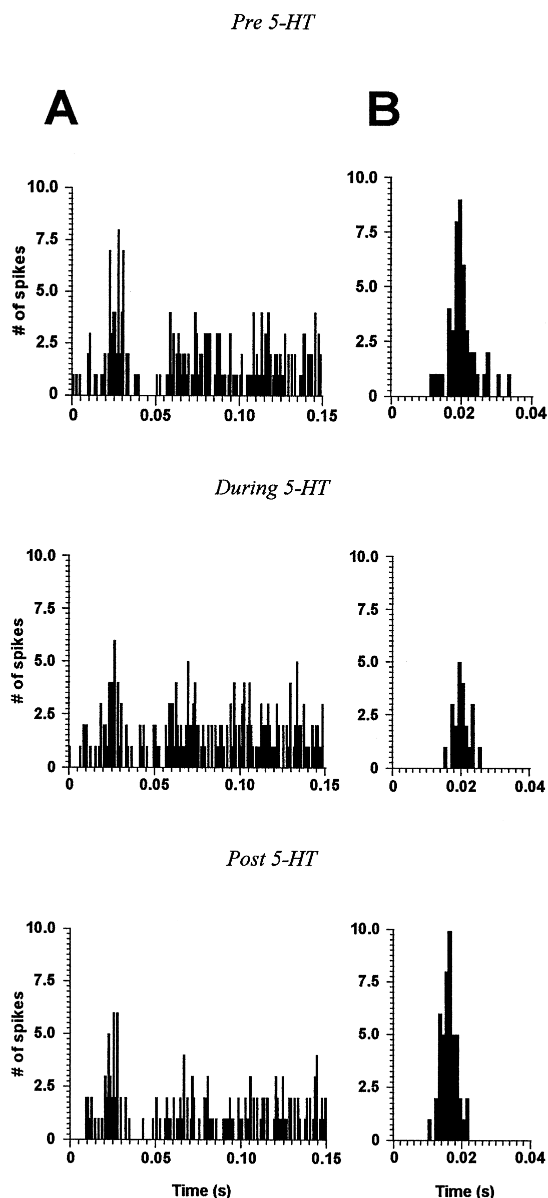

Fig. 5A, B Post-stimulus time histograms (PSTHs) calculated from unitary discharges of two RN neurones in response to somatosensory cortex electrical stimulation. The same number of trials was recorded during a control interval (*Pre 5-HT*), a 5-min microiontophoretic application of 5-HT (*During 5-HT*) and a recovery period (*Post 5-HT*). **A** The excitatory-inhibitory pattern (80 trials, 150 ms) evoked by cortical stimulation is abolished during 5-HT application (intensity 40 nA), mostly for an enhancement of the background firing. **B** The excitatory pattern (60 trials, 40 ms) evoked by cortical stimulation is depressed by 5-HT application (intensity 40 nA). Note that in both neurones the effect is reversible

calculated from 60–80 trials during cortical stimulation, were compared with those recorded in an equal number of trials during continuous microiontophoretic application of 5-HT (intensity: 20–60 nA).

In 11 RN neurones, cortical stimulation induced short-latency responses: inhibitory in six units, excitatory in two and biphasic (excitation followed by inhibition) in three cases. The effects of microiontophoretic 5-HT on the excitatory responses were studied. Long-lasting (5 min) application of 5-HT during cortical stimulation significantly attenuated (two-tailed *t*-test, $P<0.05$) the peak of the excitatory responses with respect to the background, the effect being reversible and dose-dependent. The suppression of the excitatory peak by 5-HT was due either to an enhancement of the background firing rate that "masked" the response (Fig. 5A) or to a reduced firing in response to cortical activation (Fig. 5B). In all cases excitatory responses were reduced to 50–60% of the control, but never abolished. Thus, the effects of 5-HT on excitatory responses evoked by cortex stimulation or microiontophoretic glu application were similar.

Discussion

These results demonstrate the depressive effect of 5-HT on the glu-evoked responses in RN neurones. This action is independent of the direct modulation exerted by 5-HT on the background firing rate of RN neurones and in fact is observed in units whose basal firing is enhanced, reduced or unaffected by 5-HT.

In the RN, where both 5-HT_1 and 5-HT_2 receptors have been detected (Pazos and Palacios 1985; Pazos et al. 1985), 5-HT increases the basal firing rate of neurones by an action on 5-HT_2 receptors and, less frequently, decreases the firing rate by acting on 5-HT_{1A} receptors (Licata et al. 1995).

The attenuation of the glu-evoked neuronal responses during 5-HT ejection appears likely to be mediated by 5-HT_{1A} receptors, at least partially. In fact mts, mostly a 5-HT_2 antagonist (Zifa and Fillion 1992), was able to block the 5-HT-evoked enhancement of background firing but was slightly effective in modifying 5-HT-evoked depression of glu responses. In contrast, NAN-190, a 5-HT_{1A} antagonist, attenuated the action of 5-HT on glu responses.

An involvement of 5-HT_{1A} receptors in reducing depolarisation induced by excitatory amino acids has already been demonstrated in rat locus coeruleus (Charléty et al. 1991) and neocortex (Rahman and Neuman 1993). Furthermore, a presynaptic inhibition of glutamatergic transmission mediated through activation of 5-HT_{1A} receptors has been described in the rat spinal cord (Singer et al. 1996). A similar mechanism might be considered also in our case. Presynaptic actions of 5-HT should, however, account for the RN responses evoked by cortical stimulation whereas they appear less probable in the experimental procedure involving direct glu application on RN neurones. As the effects of 5-HT on cortical and on glu responses were similar, the interaction of 5-HT and glu at a postsynaptic level seems probable although a participation of presynaptic mechanisms cannot be excluded.

On the other hand, a direct action of 5-HT as an antagonist at the same receptor site as glu appears unlikely. In fact, 5-HT, even when ejection currents were increased, was unable to block the glu-evoked responses entirely. In addition, a high positive correlation was found between the attenuation induced by 5-HT on the glu-evoked responses and the initial intensity (M and C values) of the same responses. In other words, 5-HT attenuated large glu-evoked responses more than small ones. These results do not appear compatible with a mechanism of competitive antagonism and so they allow exclusion of the possibility that 5-HT may act as a glu antagonist in the RN.

Interactions between excitatory amino acids and 5-HT have been described in various brain areas and in different animals, the most common effect of 5-HT being an attenuation of the glu-evoked response. In fact a suppressive action of 5-HT was detected in cerebellar cortex (Lee et al. 1986; Hicks et al. 1989), cerebellar deep nuclei (Gardette et al. 1987), locus coeruleus (Aston-Jones et al. 1991), substantia nigra (Aghajanian and Bunney 1975) and nucleus accumbens (White et al. 1994), whereas an enhancement of amino-acid-evoked responses was observed in facial (McCall and Aghajanian 1979) and spinal (White and Neuman 1980) motoneurones. In some structures, such as neocortex (Nedergaard et al. 1987), ventrobasal thalamus (Eaton and Salt 1989) or dorsal spinal cord (Murase et al. 1990), 5-HT induced mixed effects, whose characteristics depended on the doses of ejected 5-HT and/or on the type of amino acid receptors involved.

Our data indicate that also in the RN, although the action of microiontophoretic 5-HT on the glu responses is inhibitory, this amine is able to exert a different influence on glu-evoked responses mediated by different glu receptors. In fact, 5-HT attenuated quis-evoked responses, although to a lesser extent than glu-evoked ones, but induced mixed effects on NMDA-evoked excitations. The latter, during 5-HT ejection, were decreased in the majority of units, but enhanced in a significant number of them, mostly those located in the caudal RN zones.

Both NMDA and non-NMDA receptors are present in the RN of various species such as turtle (Keifer and Houk 1991), cat (Davies et al. 1986) and rat (Billard et al. 1991). The hypothesis that 5-HT exerts a different modulation on the excitatory neuronal responses mediated by these two types of receptors is plausible. In fact, in the presence of 5-HT a selective enhancement of the responses mediated by NMDA receptors has been described in cat and rat neocortex (Nedergaard et al. 1978; Reynolds et al. 1988, Rahman and Neuman 1993). However, in these neurones 5-HT had no effect on the excitatory responses mediated by non-NMDA receptors. A similar selective action of 5-HT, although opposite in nature, was observed in the dorsal horn of the spinal cord (Murase et al. 1990), where 5-HT depressed the effects mediated by NMDA receptors. In cerebellar cortex and deep nuclei

the modulatory action of 5-HT was depressive on both quis- and NMDA-evoked excitations, but significantly stronger on the former (Gardette et al. 1987; Hicks et al. 1989). On the other hand in ventrobasal thalamus (Eaton and Salt 1989) and in locus coeruleus (Charléty et al. 1993), 5-HT influenced quis- and NMDA-mediated excitations to a similar extent, although in the locus coeruleus 5-HT attenuated both quis- and NMDA-mediated responses but enhanced kainate-evoked effects.

In our experiments 5-HT was able to induce both effects – an enhancement or a depression of NMDA-mediated responses – in different RN neurones. A plausible hypothesis is that different mechanisms, and specifically different 5-HT receptors, are implicated in the two cases. In addition, a selective modulation of excitatory amino-acid-induced responses is suggested by the fact that in a significant number of RN neurones, tested for both NMDA and quis, 5-HT influenced the two responses differently. Further experiments with selective antagonists of 5-HT and glu receptors are necessary to clarify these aspects.

Although 5-HT modulates RN neuronal responsiveness in several ways, and interacts with more than one type of excitatory amino acid receptor, the effect of this drug on glu-evoked effects appears depressive in almost all cases. In fact glu-evoked responses were similar to the excitations mediated by non-NMDA receptors and the action of 5-HT depressed both in a similar way. Our hypothesis is that, in our experimental set-up, microiontophoretic glu evokes excitatory responses mostly via non-NMDA receptors and induces a slight involvement of NMDA receptors, which in RN neurones are segregated on distal dendrites (Davies et al. 1986; Billard et al. 1991). Furthermore glu is known to act mostly on non-NMDA receptors in the RN as well as in other nervous structures (McLennan et al. 1981; Watkins and Evans 1981; Billard et al. 1991).

Functional implications

Various data support the hypothesis that 5-HT plays an important role in processing the afferent input to the RN. In fact, in rat RN, evidence exists of a high concentration of 5-HT (Palkovitz et al. 1974) and serotonergic terminations (Steinbusch 1984), of a dense projection to the RN from the dorsal raphe (Pierce et al. 1976; Bernays et al. 1988) and of an intermediate number of 5-HT_1 and 5-HT_2 receptors (Pazos and Palacios 1985; Pazos et al. 1985).

Cerebellar nuclei provide the main bulk of glutamatergic afferents to the RN (Massion 1967; Bernays et al. 1988). These fibres, together with fewer cholinergic projections, are delivered to the soma and proximal dendrites of RN neurones in the cat (Song et al. 1993) and to the soma and the entire dendritic tree in the rat (Caughell and Flumerfelt 1977; Flumerfelt and Caughell 1978; Nauss et al. 1985). In any case this input is the strongest excitatory drive to RN neurones and acts mainly via non-NMDA receptors (Billard et al. 1991). Our results demonstrate that enhanced levels of 5-HT, corresponding to the firing of serotonergic afferens to the RN, are able to reduce the effectiveness of cerebello-rubral transmission and thus to uncouple te RN from its most important input. A similar depression of responsiveness of RN neurones to stimulation of the brachium conjunctivum has been described in the baboon in the presence of biogenic amines such as dopamine, noradrenaline and 5-HT (Huffman and Davis 1977).

According to the hypothesis advanced by Jacobs and Fornal (1993), 5-HT exerts a diffuse facilitatory action on motor output while it inhibits the processing of sensory information. The enhancement of neuronal background firing, induced by microiontophoretic application of 5-HT to motoneurones (McCall and Aghajanian 1979; Takahashi and Berger 1990) and in motor structures such as the RN (Licata et al. 1995) and the lateral vestibular nucleus (Licata et al. 1990), is in agreement with this hypothesis. On the other hand, mixed or inhibitory responses were recorded in motor cortex (Jordan et al. 1972), cerebellum (Strahlendorf et al. 1984; Gardette et al. 1987), and medial and superior vestibular nuclei (Licata et al. 1993a, b). Our results show that in the RN although the neuronal background firing rate, i.e. the noise level, increases in the presence of 5-HT, the function of the nucleus as a relay station of motor information is impaired or at least reduced.

Another important drive to the RN mediated by excitatory amino acids, even if weaker than the cerebellorubral one, is provided by corticorubral fibres. This input shows various characteristics in different animals. In fact this afferent path is a direct one in the cat, although terminations end on distal dendrites and are confined to the parvocellular zone (Pizzini et al. 1975). In the rat the two segments of the RN are not histologically distinct and the existence of a direct corticorubral path is not certain (Brown 1974; Nauss et al. 1985). The few excitatory responses we recorded in RN neurones upon cortical stimulation were significantly depressed by microiontophoretic 5-HT. This effect appeared to be due more to an enhancement of the background activity than to an effective decrease of the response. Therefore, even if the action of 5-HT on the weak cortically induced excitations was inhibitory, it appeared to be smaller than the strong influence described on the excitations induced by stimulation of brachium conjunctivum (Huffman and Davis 1977).

On the whole 5-HT is in a position to selectively gate or filter the motor glutamatergic input to the RN, depressing the short-term responses that are thought to be mediated by non-NMDA receptors.

The present findings indicate a different serotonergic modulation of NMDA-mediated effects and the segregation of the enhancing and depressive effects of 5-HT in the caudal and rostral zones respectively. It is worth moting that in mammals the population of RN large cells is located in the caudal part of the nucleus only. The two divisions are not histologically clearly distinct in the rat (Brown 1974), but it cannot be excluded that the different

behaviour of 5-HT on NMDA responses results from its effects on two different population of RN neurones (e.g. local interneurones and projecting neurones, or rubrospinal and rubro-olivary neurones). Furthermore, as NMDA receptors are also involved in long-term potentiation, the influence of 5-HT on these receptors deserves attention and better understanding.

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