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Electrophysiological and Pharmacological Characterization of Serotonergic Dorsal Raphe Neurons Recorded Extracellularly and Intracellularly in Rat Brain Slices

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Extracellular and intracellular recordings were made from dorsal raphe (DR) neurons in frontal rat brain slices maintained in vitro. A population of neurons was found which displayed electrophysiological and pharmacological characteristics of serotonin-containing DR neurons recorded in vivo. Recorded extracellularly, these neurons displayed biphasic or triphasic action potentials of 1.5–3.0 ms duration, and discharged with a slow and steady rhythm. Recorded intracellularly these neurons displayed action potentials of about 1.8 ms duration, which were followed by large (10–20 mV) afterhyperpolarizations which normally lasted 200–800 ms. These presumed serotonergic DR neurons were inhibited by LSD and serotonin. They were excited by norepinephrine, or the α-agonist phenylephrine, and these activations could be reduced or blocked by α-adrenoreceptor antagonists including the selective α_1-antagonist, prazosin. The major difference between the in vitro recordings and previous in vivo recordings from anesthetized animals was a reduction in the number of spontaneously firing DR neurons. This was probably due, at least in part, to a disfacilitation of serotonergic DR neurons in the slice caused by the functional removal of a tonic noradrenergic input.

INTRODUCTION

The dorsal raphe (DR) nucleus contains the largest cluster of serotonin-containing neurons in the rat brain[13,28]. However, because non-serotonergic as well as serotonergic neurons are present in the DR nucleus[7,29] it is useful in single-cell recording studies to be able to recognize the serotonergic neurons on the basis of their electrophysiological and pharmacological characteristics. Such a characterization has already been made for serotonergic dorsal raphe neurons recorded in vivo, using either extracellular or intracellular recording techniques in combination with selective lesioning of serotonergic neurons[7], antidromic stimulation of an identified serotonergic pathway[7,35], visualization of serotonin-containing histofluorescent neurons in the immediate vicinity of the recording electrode[2], and direct intracellular injection of a dye into electrophysiologically characterized presumed serotonergic neurons, and the subsequent visualization of both the injected dye and serotonin histofluorescence inside the recorded neurons[4]. In these previous studies, serotonergic DR neurons have been shown to discharge in a slow and steady manner, with rates usually below 2.5 Hz. In addition, these serotonergic DR neurons have been pharmacologically characterized as being inhibited by serotonin, and even more potently by LSD[1,3,16]. The spontaneous firing of these neurons in anesthetized rats[8,11,24,30] and cats[32] appears to be largely dependent upon a tonic noradrenergic synaptic input, since they lose most of their spontaneous activity when the noradrenergic input to them is either reduced by reserpine pretreatment[11] or blocked by α-adrenoreceptor blockers[8,9,23,24]. Nevertheless, at least some dorsal raphe neurons are still spontaneously active in the isolated brain slice preparation in which most of the afferent connections to these neurons, including the noradrenergic input, have been cut[25,31].

The purpose of the present study was 4-fold: (1) to replicate the previous in vitro extracellular recording studies[25,31] showing spontaneously active DR neu-

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rons; (2) to examine some of the intracellular characteristics of DR neurons in the brain slice; (3) to characterize physiologically and pharmacologically the serotonergic neurons of the dorsal raphe nucleus in this preparation and to distinguish them from non-serotonergic neurons present in this nucleus[7,29]; and (4) to study in a quantitative manner the α-adrenergic influences on the firing of serotonergic dorsal raphe neurons. This last goal was more easily accomplished using the in vitro brain slice preparation since known concentrations of α-adrenoreceptor agonists and antagonists could be introduced into the perfusion solution in the absence of any anesthetic agents. Preliminary results of this study have been presented[34].

METHODS

A total of 68 albino rats (Charles River Laboratories) was used for this study. Most of the data was obtained from male rats weighing from 100–300 g, although in some of the early experiments rats of either sex, weighing as little as 50 g were also used. No differences in the results were noted.

For all extracellular recording experiments, rats were anesthetized with halothane (Fluothane, Ayerst Labs, Inc.) prior to decapitation. For some of the intracellular recording experiments, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and allowed to breath 100% O_2 for 5 min before decapitation. This procedure appeared to improve the quality of intracellular recordings. Following removal of the brain, frontal sections, nominally 450 μm in thickness, were cut through the dorsal raphe nucleus using a Sorvall tissue sectioner (model TC-50), and transferred to a brain slice chamber modified from the design of Haas et al.[15]. The procedure took from 2.5 to 4.5 min from decapitation to placing the slices in the chamber. The brain slices rested upon a piece of Kimwipe tissue, (Kimberly-Clark) or a piece of lens tissue (Macalaster Bicknell, #32215), through which flowed a Ringer's solution (artificial cerebrospinal fluid) of the following composition in mM; NaCl, 130; KCl, 5.0 or 6.25; $CaCl_2$, 2.0 or 4.0; $MgSO_4$, 1.5 or 3.0; $NaHCO_3$, 24; NaH_2PO_4, 1.25; D-glucose, 10.0. Ca^{2+} concentrations were varied in some experiments to ascertain calcium's effects on spontaneous activity and other neuronal properties. The Kimwipe or lens tissue rested upon a piece of netting (nylon or dacron) which was raised about 3 mm above the floor the chamber. Humidified 95% O_2–5% CO_2 gas circulated both above and below the netting. For most intracellular, and some extracellular experiments a layer of surgical gauze was placed over the slices. This caused a thin fluid layer of Ringer's solution to flow over the slices and prevented drying of the slice surface. Recordings were made in the gaps between the gauze threads, which could be gently moved aside if necessary. The temperature of the chamber was maintained at 35.0–36.5 °C, except for some intracellular recording experiments in which the chamber temperature was allowed to drop to 31 °C. This was done to see if lower temperatures improved intracellular recording conditions, but no consistent advantages of lowered temperature were found in the present study.

Various drugs were dissolved, in known concentrations, in the Ringer's solution and introduced into the slice chamber by a stopcock arrangement. Almost complete exchange of fluids occurred within five minutes of the arrival of the new solution in the chamber. Norepinephrine solutions, which were subject to oxidative deterioration, were used within 2 h of preparation.

For intracellular recordings, glass microelectrodes were pulled on a Narishige vertical puller (Model PE-2) from 2.0 mm o.d. 'omega dot' tubing (W.P.-Instruments), and filled with either KCl (1 or 2 M) or potassium acetate (4 M). They had resistances of 40–90 MΩ. Standard intracellular recording techniques similar to those previously described[4,5] were employed, using a W.P.-Instruments M707 amplifier, Tektronix oscilloscopes, a Gould Brush pen recorder (Model 220), and a Hewlett Packard FM tape recorder (model 3964A, frequency response 0–1250 Hz) to store data.

Extracellular single-unit recordings were made using single-barrel or 5-barrel (iontophoretic) glass micropipettes. Recording electrodes were filled with 2 M NaCl saturated with Fast Green dye, or with 1 M NaCl, and had resistances of 4–14 MΩ. For microiontophoresis, the side barrels of 5-barrel micropipettes were filled with various combinations of the following solutions: L-norepinephrine (NE, Regis, 0.1 M, pH 4.0), D-lysergic acid diethylamide (LSD, PHS-NIDA, 1.0 mM in 0.1 M NaCl, pH 4.0); L-phenylephrine-HCl (PE, Sigma, 0.1 M, pH 4.0); L-glutamate

monosodium (Sigma, 0.1 M, pH 8.0). One of the side barrels always contained 2 M NaCl for automatic current balancing[27]. All drugs were ejected using positive current, except for glutamate, which required negative current. Drugs were retained between periods of ejection by passing 10–20 nA current of the opposite polarity as used for ejection.

For all recordings, the tip of the electrode was precisely placed in the DR nucleus, which was easily visualized in the slices using a stereomicroscope (Wild, Model M-5). As described in the Results section, extracellular and intracellular recordings were obtained from neurons in the brain slice which fulfilled the electrophysiological and pharmacological criteria for serotonergic DR neurons recorded in vivo. These recordings were presumed, therefore, to be from the serotonergic DR neurons in the brain slices.

RESULTS

Extracellular electrophysiological characterization of presumed serotonergic DR neurons

Fig. 1 shows some examples of extracellular single-unit recordings made from presumed serotonergic DR neurons in brain slices. As is the case for in vivo recordings, these neurons discharged with a slow and steady rate (Fig. $1C_2$), with rather broad action potentials (1.5–3.0 ms). Serotonergic DR neurons recorded extracellularly in vivo display a mainly biphasic, positive–negative action potential[7,35,36], although recent careful observations have revealed the presence of triphasic, positive-negative-positive spikes as well (Aghajanian, unpublished data). Presumed serotonergic DR neurons recorded in vitro in the present study displayed biphasic (Fig. 1A, B) and

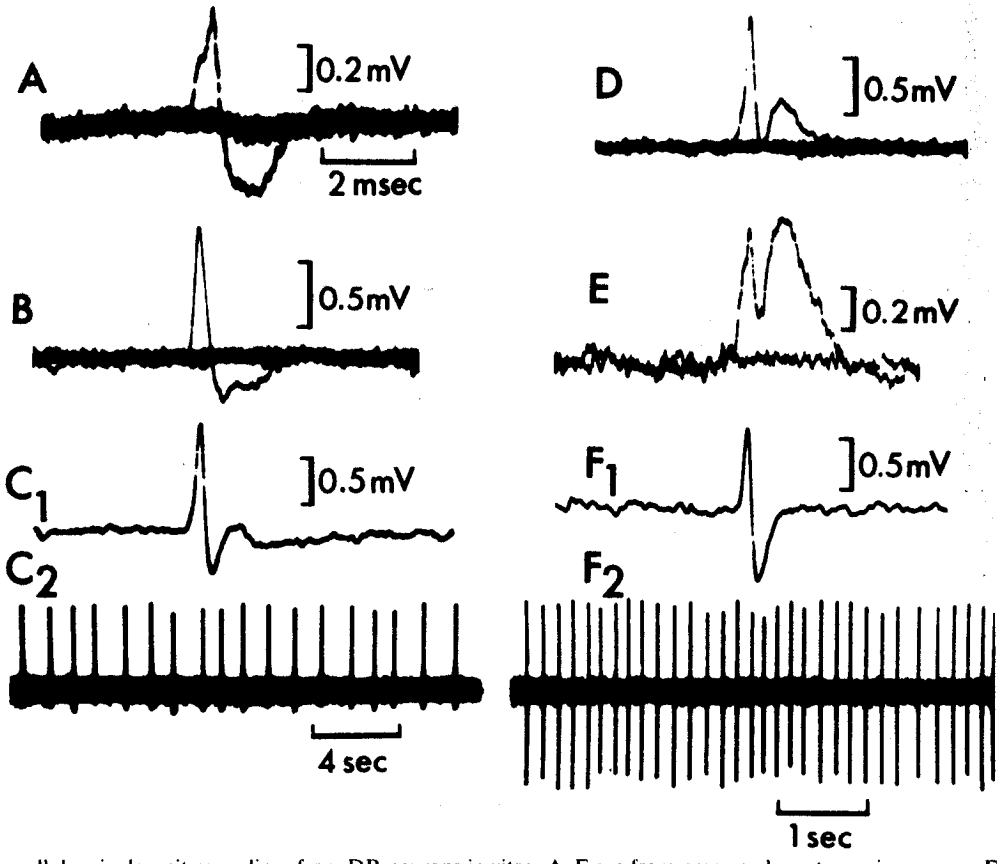

Fig. 1. Extracellular single-unit recordings from DR neurons in vitro. A–E are from presumed serotonergic neurons; F is from a presumed non-serotonergic neuron. C_1 and C_2 are from the same neuron recorded at different oscilloscope sweep speeds, as is the case for F_1 and F_2. Notice the continuum of waveform variations for presumed serotonergic neurons from biphasic (A), to biphasic with a slight bump on the negative component (B), to triphasic (C_1), to 'double-bump' (D and E). Calibration in A is 2 ms and is for all traces except C_2 and F_2.

triphasic (Fig. $1C_1$) spikes, as well as a variation of the triphasic spike consisting of a dual positivity or 'double-bump' (Fig. 1D, E). Other characteristics, such as total duration of spike, discharge rate and pattern[6,7], and response to drugs such as LSD[3] and serotonin[16], were entirely typical of serotonergic DR neurons recorded in vivo. The different extracellular spike shapes observed in the present study are really part of a continuum ranging from biphasic to triphasic to double-bump. In some instances transformations from one waveform variation to another were observed while recording from a single neuron, with no indications of concomitant injury or irritation to the recorded cell. Variations in spike waveform could often be observed for individual neurons simply by moving the recording electrode up or down a few micrometers in the tissue.

Most of the viable serotonergic neurons in the brain slice studies here were not spontaneously active. This was verified in experiments in which iontophoretically applied glutamate was used to activate quiescent cells (e.g. Fig. 7B) or in which either norepinephrine or phenylephrine (as will be described later) was used to cause silent cells to fire (e.g. Figs. 3B, 4 and 7). For example, for two slices from two different rats, the average number of spontaneously active serotonergic neurons encountered per electrode tract was 0.3 (n = 20 tracts) using a single-barrel electrode and an extracellular calcium concentration of 2.0 mM. When phenylephrine (10–20 μM) was included in the perfusion medium, a search for spontaneously active serotonergic neurons in the same areas of the DR nucleus yielded an average of 1.35 cells per tract (n = 20 tracts). These recordings were done in slices covered with gauze (see Methods) to prevent drying of the surface of the slices. In other experiments it was found that raising the extracellular Ca^{2+} concentration to 4.0 mM virtually abolished spontaneous activity of presumed serotonergic DR neurons. A more detailed account of the effects of varying extracellular calcium concentrations on DR neuronal properties will be given in a separate report (VanderMaelen and Aghajanian, in preparation).

Non-serotonergic DR neurons

A second population of extracellularly recorded units was encountered in the DR nucleus in the brain slice. These units displayed action potentials of brief duration (1 ms or less), which were usually biphasic positive-negative, with both components of approximately equal amplitude (Fig. $1F_1$). When spontaneously active, they often fired at rates 2 or 3 times faster than presumed serotonergic neurons (Fig. $1F_2$), and were capable of discharging at rates in excess of 20 Hz when driven by iontophoretically applied glutamate. In contrast, presumed serotonergic DR neurons were incapable of sustained firing in excess of 10 Hz when driven by iontophoretically applied glutamate. These short-duration extracellular units, which can also be recorded in vivo[7], were presumed to be non-serotonergic DR neurons. Besides their markedly different electrophysiological characteristics, these presumed non-serotonergic DR neurons were pharmacologically distinguishable by their relative lack of responsiveness to iontophoretically applied serotonin and LSD, and by a mild depression rather than an excitation, in response to NE.

Intracellular recordings from presumed serotonergic DR neurons

Intracellular recordings of acceptable quality were made from 34 DR neurons in slices from 18 rats. Recordings were deemed acceptable if the action potential was at least 60 mV in amplitude, and the input resistance, measured by means of injected constant current hyperpolarizing pulses, was at least 40 MΩ. Average spike amplitude was 69 mV (range = 60–84 mV). Average input resistance was 94 MΩ (range = 40–230 MΩ), with most measurements being higher than those generally observed for serotonergic DR neurons recorded in vivo[5].

Except for the somewhat higher input resistance measurements, these intracellular recordings in vitro resembled in every way intracellular recordings made from positively identified serotonergic DR neurons in vivo[4]. Therefore, these recordings were assumed to be from the serotonergic DR neurons in the slice.

Spontaneously active presumed serotonergic DR neurons recorded intracellularly in brain slices discharged in a slow and steady manner. Silent neurons could be made to fire in a slow and steady manner by continuous intracellular injection of depolarizing current (0.05–0.4 nA), or by adding phenylephrine (100 μM) to the Ringer's solution. Each action potential was followed by a prominent afterhyperpolariza-

tion of 10–20 mV (measured from spike threshold, Fig. 2, $\bar{x}$ = 15.7 mV; S.D. = 3.3 mV, n = 19 neurons). Close examination of slowly firing (about 1 Hz) cells revealed that each afterhyperpolarization decayed gradually over a 200–800 ms period, after which the membrane potential tended to plateau for another couple of hundred milliseconds (e.g. Fig. $2A_1$). Following this short plateau phase a slight depolarization of the membrane usually preceded the triggering of the next spike, which then began the cycle anew. In more rapidly firing neurons the decay of the afterhyperpolarization was accelerated, and the plateau phase was reduced or absent (e.g. Fig. 6).

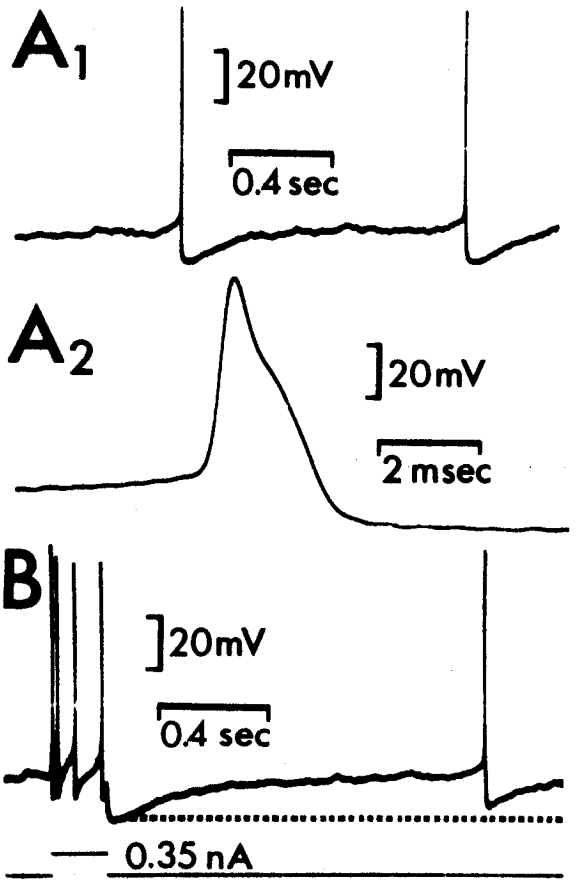

Fig. 2. Intracellular recordings from presumed serotonergic DR neurons in vitro. A_1 and A_2 are from the same neuron, and B is from another neuron. A_1: spontaneous action potentials. Notice the large afterhyperpolarization which follows each spike. A_2: a spontaneous action potential seen with faster oscilloscope sweep. Notice the slight shoulder on the falling phase of the spike. B: a burst of spikes induced by an intracellularly injected current pulse (0.35 nA) is followed by an afterhyperpolarization which is larger than the afterhyperpolarization which follows the spontaneously occurring spike which fires about 1.4 s after the burst.

Although these neurons did not normally fire in bursts, a burst of spikes could be induced by intracellular injection of a depolarizing current pulse. Such a burst of spikes was followed by an afterhyperpolarization which was larger than hyperpolarizations following single spikes (Fig. 2B), as well as by a post-activation inhibition of spontaneous activity (if present), which was longer than the normal interspike interval. These characteristics are identical to those previously observed for serotonergic DR neurons recorded in vivo[4,5].

Spontaneously occurring intracellularly recorded action potentials were approximately 1.8 ms in duration ($\bar{x}$ = 1.83 msec, n = 5 neurons) when measured straight across from the point at which the rapid upstroke of the spike began, and exhibited a slight shoulder on their falling phase (Fig. $2A_2$).

Occasionally a second type of neuron in the DR was impaled. These cells displayed short duration (1 ms) action potentials, and lacked prominent spike afterhyperpolarizations. These cells were assumed to be non-serotonergic DR neurons, and were not investigated further in this study.

Responses of presumed serotonergic DR neurons to LSD and serotonin

As is the case for serotonergic DR neurons recorded in vivo[3,16], presumed serotonergic DR neurons in brain slices were inhibited by very low currents of iontophoretically applied LSD (Fig. 3A), and by low currents of serotonin (Fig. 3B). Serotonin also suppressed activations of neuronal firing induced by iontophoretically applied NE (Fig. 3B), or bath application of phenylephrine (Fig. 7B). Inhibition was also observed following bath application of LSD (50–200 nM).

Responses of presumed serotonergic DR neurons to norepinephrine, phenylephrine, and α-adrenergic antagonists

All presumed serotonergic DR neurons tested in the slice could be excited by norepinephrine (NE) or the α-adrenoreceptor agonist phenylephrine (PE), when applied either by iontophoresis (currents = 2–15 nA) or in the perfusion medium (concentrations = 1–400 μM). Some examples of this are illustrated in Fig. 4. Such activations occurred for cells which were already spontaneously firing (e.g. Fig. 4B), as

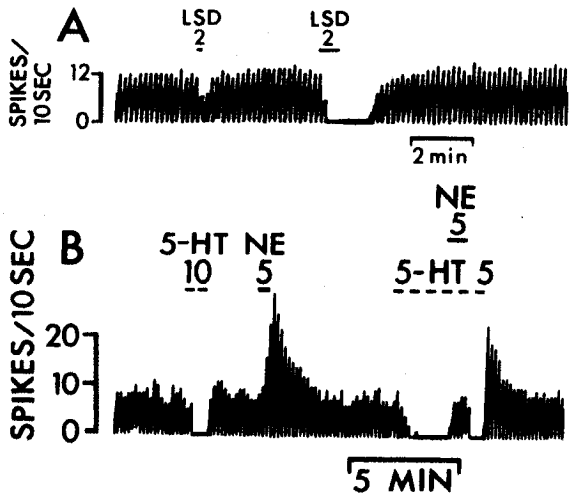

Fig. 3. Inhibition of firing of presumed serotonergic DR neurons by LSD and serotonin (5-HT). These are extracellular single-unit recordings displayed in ratemeter form, in which the height of each line represents the number of spikes which fired during a 10-s period of time. A: LSD administered iontophoretically (2 nA) from a 5-barrel micropipette during the time indicated by the bar inhibited the spontaneous firing of this neuron. B: 5-HT administered iontophoretically during the time indicated by the broken lines inhibited the spontaneous firing of this neuron (left side), and also greatly reduced the absolute amount of activation induced by iontophoretically applied NE (right side). Currents are in nA.

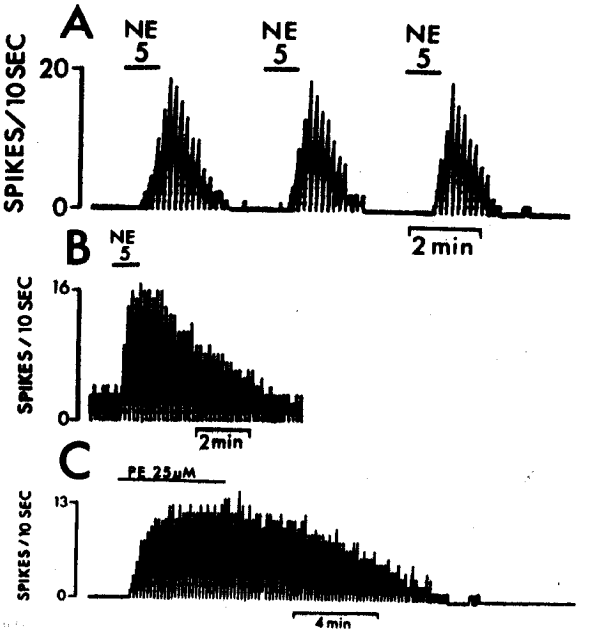

Fig. 4. Activations of presumed serotonergic DR neurons by norepinephrine (NE) and the α-adrenoreceptor agonist phenylephrine (PE). A: a neuron which was not spontaneously active was repeatedly made to fire by iontophoretic administration of NE (5 nA). B: a spontaneously active neuron was further activated by iontophoretically applied NE (5 nA). C: a neuron which was not spontaneously active was made to fire by the inclusion of PE (25 μM) in the perfusion medium during the time indicated by the bar. The slight delay in response onset was due mainly to the delay between switching the solution flowing into the chamber, and the arrival of the PE at the slice.

well as for neurons which were silent (e.g. Fig. 4A, C). Silent cells could be found with 5-barrel iontophoretic electrodes by ejections of NE, or the excitatory amino acid glutamate (e.g. Fig. 7B). With single barrel electrodes, silent cells could be found by adding PE to the perfusion medium (e.g. Fig. 7A).

The maximum firing rate attained by a presumed serotonergic DR neuron in response to bath-applied phenylephrine was approximately 6 Hz. But most cells responded to NE or PE with firing rates between about 1 and 3.5 Hz, which is within the range of spontaneously firing serotonergic DR neurons recorded in vivo (e.g. Figs. 3B, and 4–7)[3,6].

Intracellular recordings were made from 6 presumed serotonergic DR neurons while phenylephrine was added to the perfusion medium. In those instances PE (100 μM) induced an increase in the spontaneous discharge rate (n = 3) (Fig. 6), or caused silent neurons to begin firing (n = 3). A relatively high concentration (100 μM) of PE was used in order to see rapid and maximal effects of the drug. No prominent changes in spike threshold, spike amplitude, or initial amplitude of afterhyperpolarization were observed to be associated with PE-induced activations. The duration of the spike afterhyperpolarization was, however, reduced (Fig. 6).

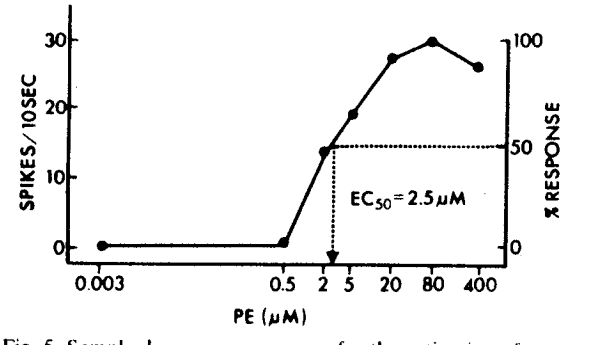

Fig. 5. Sample dose–response curve for the activation of a presumed serotonergic DR neuron by phenylephrine (PE), applied in the perfusion medium. Concentrations were presented in ascending order, with ample time (12 min) allowed for equilibration of the response at each concentration.

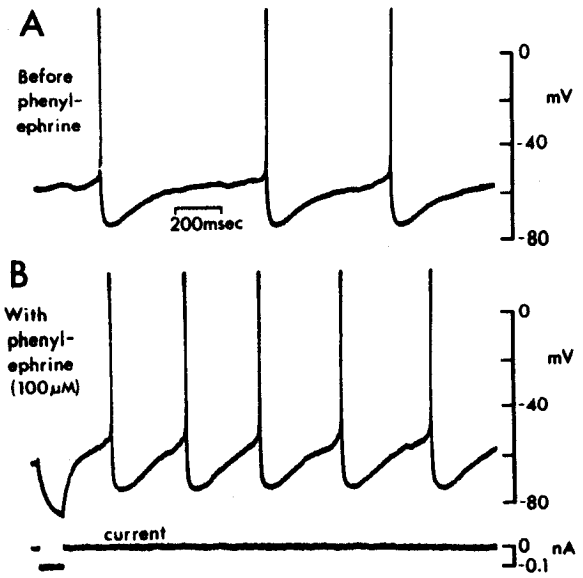

Fig. 6. Intracellular recordings from a presumed serotonergic DR neuron before (A) and during (B) the bath application of phenylephrine (100 μM). The spontaneous discharge rate in A was about 1.5 Hz. This was approximately doubled by the phenylephrine. The decay of the spike afterhyperpolarization was accelerated during the response to phenylephrine, but the amplitude of the afterhyperpolarization was unchanged. Intracellularly injected current pulse in B (0.1 nA) indicated input resistance of about 210 MΩ (but this varied as a function of time since the last spike).

Individual dose–response curves for activations of presumed serotonergic DR neurons by PE were constructed (n = 6 neurons). Estimated EC_{50}s ranged from 2.0 to 6.5 μM (geometric mean = 3.9 μM). A sample dose–response curve for PE is shown in Fig. 5. When NE and PE were bath-applied in equal concentrations to the same neurons, the responses to NE were always smaller than for PE by about 50% (n = 4 neurons).

Activations of presumed serotonergic DR neurons by NE or PE could be partially or totally blocked by bath application of the α-adrenoreceptor antagonists phentolamine (15–20 μM, n = 5 neurons, e.g., Fig. 7A) and thymoxamine (15 μM, n = 3 neurons), including the selective α_1-adrenoreceptor antagonist prazosin (20 nM–10 μM, n = 9 neurons, Fig. 7B).

DISCUSSION

The present study has shown that both extracellular and intracellular recordings can be made from presumed serotonergic DR neurons in isolated rat brain slices maintained in vitro. This is the first report of intracellular recordings from these neurons in a brain slice preparation. As summarized in Table I, in almost all respects, these neurons resemble serotonergic DR neurons recorded in vivo from anesthetized rats. For example, as with in vivo recordings, spontaneously firing presumed serotonergic DR neurons were encountered in the present study. This is in good agreement with the initial study by Mosko and Jacobs[25] who also recorded from rat brain slices, as well as a recent report by Trulson et al.[31], in which mouse brain slices were used. However, unlike the situation in vivo in which the great majority of serotonergic DR neurons are spontaneously active[6], most viable DR neurons in the present experiments were silent in the slice. This finding would also appear to be in agreement with the earlier study of Mosko and Jacobs[25] who found a total of 21 spontaneously active units in 21 rats. Although early technical difficulties associated with the brain slice preparation could account for this low yield, it is possible that many healthy but silent DR neurons were present in their slices as well. A possible explanation for the reduced level of spontaneous activity in these neurons is that all excitatory afferents to the nucleus which originate outside of the slice have been cut. One such afferent system is the norepinephrine system, which has been shown to make direct synaptic contacts onto serotonergic DR neurons[10]. Since norepinephrine excites

TABLE I

Comparison of in vivo and in vitro recordings from serotonergic dorsal raphe neurons in rats

Information on in vivo recordings was obtained from various studies, referenced in the text, performed on chloral hydrate-anesthetized rats. Information on in vitro recordings is from the present study

	In vivo	*In vitro*
1. Most cells spontaneously active.	yes	no
2. Long duration bi- or triphasic extracellular spike.	yes	yes
3. Slow and steady discharge.	yes	yes
4. Inhibited by LSD.	yes	yes
5. Inhibited by 5-HT.	yes	yes
6. Activated by iontophoretic NE.	yes	yes
7. Post-activation inhibition.	yes	yes
8. Prominent spike after-hyperpolarizations.	yes	yes
9. Burst afterhyperpolarization larger than single spike afterhyperpolarization.	yes	yes

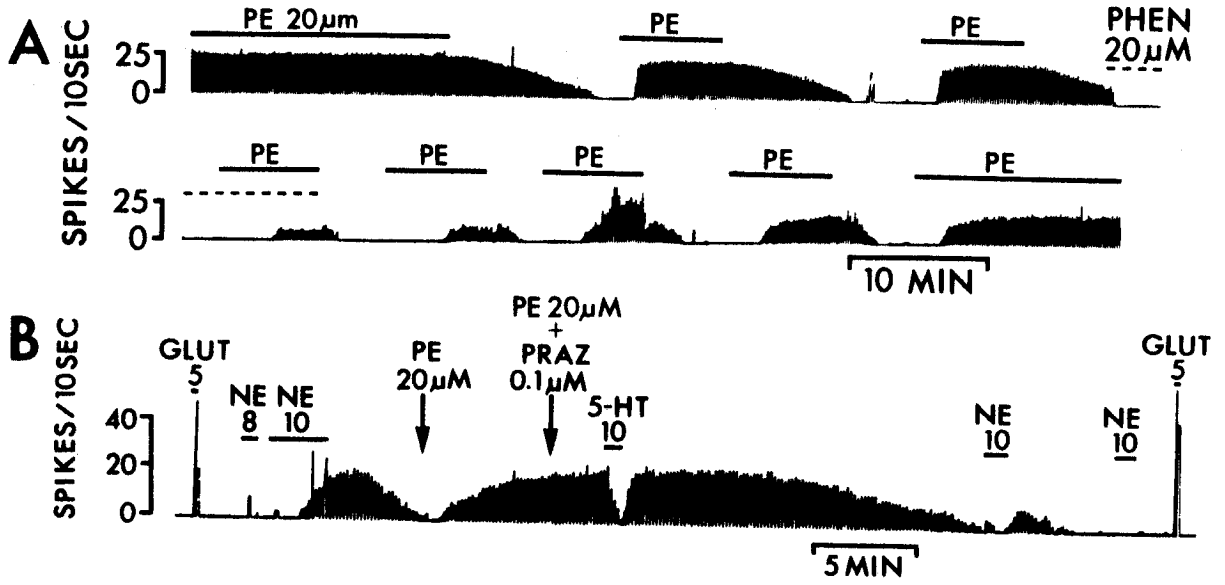

Fig. 7. Antagonism of the NE- and PE-induced activations of presumed serotonergic DR neurons by α-adrenoreceptor antagonists. A: PE (20 μM) applied in the bath during the times indicated by the solid lines repeatedly activated this neuron, which was normally silent. When phentolamine (PHEN, 20 μM) was introduced into the bath during the time indicated by the dashed line, the response to PE was greatly reduced. Partial recovery of the response to PE occurred over the next 4 applications. B: a neuron which was silent could be activated by iontophoretically applied glutamate (GLUT, 5 nA), as well as by NE (10 nA). Bath-applied PE (20 μM, beginning at arrow) also caused the neuron to fire, and this could be inhibited by iontophoretically applied serotonin (5-HT, 10 nA). When the selective α_1-antagonist, prazosin, was added to the perfusion medium, which still contained phenylephrine (PE 20 μM + PRAZ 0.1 μM), the PE-induced activation was gradually antagonized. After about 20 min this antagonism could be partially overcome by the iontophoretic application of NE (10 nA), but a few minutes later NE was also ineffective. The presence and health of the neuron, as well as a lack of local anesthetic effect, was indicated by the neuron's response to GLUT (5 nA, right-hand side of figure).

presumed serotonergic DR neurons in the brain slice (present study), and reduction or blockade of the noradrenergic input to these neurons in the anesthetized animal in vivo results in a great decrease in their spontaneous activity[8,9,11,30,32], it is likely that the reduced level of spontaneous activity of presumed serotonergic DR neurons in the present study was caused, at least in large part, by a dysfacilitation due to the functional removal of the tonic noradrenergic input to these cells.

If most serotonergic DR neurons are dependent upon extrinsic excitatory or facilitatory inputs in order to exhibit their characteristic spontaneous activity, does this contradict previous studies suggesting that these neurons may function as 'autonomous pacemakers'[25], with an 'endogenous rhythm'[31] due to the presence of 'pacemaker potentials'[5]? Such a contradiction exists only if one insists that 'endogenous rhythms' and 'pacemaker potentials' must, by definition, be totally 'autonomous', i.e. completely independent of all extrinsic synaptic or neurohumoral influences. Such a restrictive definition would, however, seem inappropriate since some invertebrate neurons display prominent and well-described 'pacemaker potentials' only when certain afferent fibers are stimulated[22] or only when exposed to certain neurohumoral substances[12,17].

Thus, based upon the present study as well as previous intracellular[5] and extracellular experiments[25,31], it appears that serotonergic DR neurons do possess pacemaker potentials, but for most of these neurons the expression of these pacemaker potentials is dependent upon the presence of adequate tonic excitatory influences. These influences may be provided by noradrenergic synaptic input[8,10,11] as occurs in vivo, or by exogenously applied α-adrenoreceptor agonists, as was done in the present study, or possibly by many other means as well. For example, intracellularly recorded serotonergic DR neurons which were quiescent in the slice could be made to fire in a slow and regular fashion simply by injecting a continuous low-amplitude (0.05–0.4 nA) depolarizing current. And in unanesthetized freely moving cats, some spontaneous activity is present in the dor-

sal raphe nucleus even after administation of high doses of α-adrenoreceptor blocking drugs, especially when the animal is aroused by sensory stimulation[18]. It would therefore appear that in unanesthetized animals there are additional excitatory or facilitatory influences operating which can maintain spontaneous activity, or at least temporarily restore it, even after pharmacological blockade of the noradrenergic input to serotonergic DR neurons[18,32]. In brain slices, on the other hand, even though no anesthesia is present, there is a reduction in both noradrenergic and other inputs to these cells (e.g. from sensory systems), and thus most of these neurons remain silent.

And finally, it should be remembered that many factors, most of them unknown, associated with the preparation and maintenance of the brain slices undoubtedly influence the amount of spontaneous activity present. For example, it was noticed that when the surface of slices was allowed to become slightly dried (see Methods) there was an increase in spontaneous activity, especially near the surface of the slice.

Extracellularly recorded action potentials from presumed serotonergic DR neurons showed considerable variations, ranging from biphasic to triphasic to 'double-bump'. Intracellularly recorded action potentials displayed much less variability, differing mainly in the amplitude of the spike afterhyperpolarization, and in the prominance of a shoulder on the falling phase of the spike. The reasons for the variations in extracellular waveform are unknown, but it is likely that they are the result of rather complex geometric relationships between the recording electrode and the cell's dendrites, soma, and initial segment[20]. This is supported by the finding that changes in the extracellular spike shape for a given neuron could usually be seen with slight changes in the position of the recording electrode.

Every presumed serotonergic DR neuron tested in the present study was activated by NE or the α-agonist PE, applied either iontophoretically or in the perfusion medium. Similarly, in vivo, most serotonergic DR neurons can be activated by low currents of iontophoretically applied NE[8]. Effective concentrations for half-maximal responses to PE in the present study were in the very low micromolar range (geometric mean = 3.9 μM, n = 6). NE was slightly less potent than PE. Since activations of DR neurons by PE and NE could be reduced or blocked completely by the α-adrenoreceptor antagonists phentolamine and thymoxamine, as well as by the selective α_1-antagonist prazosin, the present results support previous in vivo studies which concluded that noradrenergic activation of serotonergic DR neurons is via an α_1-adrenergic receptor[8,23,24].

The membrane mechanisms underlying the noradrenergic activation of serotonergic DR neurons are still completely unknown. However, the preliminary intracellular data reported in the present study do suggest that these activations are probably not caused primarily by a norepinephrine-induced decrease in a calcium conductance, G_{Ca}. Changes in G_{Ca} have been shown to occur in sympathetic ganglion cells in response to NE[14,19], and in heart muscle cells in response to epinephrine[26,33]. If a decrease in G_{Ca} was induced in serotonergic dorsal raphe neurons by α-adrenoreceptor agonists, then one would expect this to cause a corresponding decrease in any calcium-activated potassium conductance (e.g. ref. 19). Such a decrease in the calcium-activated G_K would be evident as a decrease in the amplitude of spike afterhyperpolarizations during PE-induced activations. Such decreases in spike afterhyperpolarizations did not occur (e.g. Fig. 7). However, PE (and NE) could still be affecting a calcium-activated G_K by accelerating the rate of intracellular calcium sequestration (such as with epinephrine in the heart; ref. 21), which would speed up the decay of the afterhyperpolarization and shorten the inter-spike interval. Unfortunately, at the present time the participation of a calcium-activated G_K in the pacemaker cycle of serotonergic dorsal raphe neurons has still not been conclusively demonstrated, although some evidence suggests that it may be important[5]. An adequate understanding of the underlying mechanisms responsible for the noradrenergic activation of serotonergic DR neurons, as well as the various conductances responsible for the pacemaker potentials themselves will require additional in vitro experiments utilizing intracellular recording and voltage-clamping techniques.

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REFERENCES

1 Aghajanian, G. K., Foote, W. E. and Sheard, M. H., Lysergic acid diethylamide: sensitive neuronal units in the midbrain raphe, *Science*, 161 (1968) 706–708.
2 Aghajanian, G. K. and Haigler, H. J., L-Tryptophan as a selective histochemical marker for serotonergic neurons in single-cell recording studies, *Brain Research*, 81 (1974) 364–372.
3 Aghajanian, G. K., Haigler, H. J. and Bloom, F. E., Lysergic acid diethylamide and serotonin: direct actions on serotonin-containing neurons in rat brain, *Life Sci.*, 11 (1972) 615–622.
4 Aghajanian, G. K. and VanderMaelen, C. P., Intracellular identification of central noradrenergic and serotonergic neurons by a new double labeling procedure, *J. Neurosci.*, 2 (1982) 1786–1792.
5 Aghajanian, G. K. and VanderMaelen, C. P., Intracellular recordings from serotonergic dorsal raphe neurons: pacemaker potentials and the effect of LSD, *Brain Research*, 238 (1982) 463–469.
6 Aghajanian, G. K. and Wang, R.Y., Physiology and pharmacology of central serotonergic neurons. In M. A. DiMascio and K. F. Killam (Eds.), *Psychopharmacology, A Generation of Progress*, Raven Press, New York, 1978, 171–183.
7 Aghajanian, G. K., Wang, R. Y. and Baraban, J. M., Serotonergic and non-serotonergic neurons of the dorsal raphe: reciprocal changes in firing induced by peripheral nerve stimulation, *Brain Research*, 153 (1978) 169–175.
8 Baraban, J. M. and Aghajanian, G. K., Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonists, *Neuropharmacology*, 19 (1980) 355–363.
9 Baraban, J. M. and Aghajanian, G. K., Suppression of serotonergic neuronal firing by α-adrenoceptor antagonists: evidence against GABA mediation, *Europ. J. Pharmacol.*, 66 (1980) 287–294.
10 Baraban, J. M. and Aghajanian, G. K., Noradrenergic innervation of serotonergic neurons in the dorsal raphe: demonstration by electron microscopic autoradiography, *Brain Research*, 204 (1981) 1–11.
11 Baraban, J. M., Wang, R. Y. and Aghajanian, G. K., Reserpine suppression of dorsal raphe neuronal firing: mediation by adrenergic system, *Europ. J. Pharmacol.*, 52 (1978) 27–36.
12 Barker, J. L. and Smith, T. G., Jr., Peptide regulation of neuronal membrane properties, *Brain Research*, 103 (1976) 167–170.
13 Dahlström, A. and Fuxe, K., Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons, *Acta physiol. scand.*, 62, Suppl. 232 (1965) 1–55.
14 Galvan, M. and Adams, P. R., Control of calcium current in rat sympathetic neurons by norepinephrine, *Brain Research*, 244 (1982) 135–144.
15 Haas, H. L., Schaerer, B. and Vôsmansky, M., A simple perfusion chamber for the study of nervous tissue slices in vitro, *J. Neurosci. Meth.*, 1 (1979) 323–325.
16 Haigler, H. J. and Aghajanian, G. K., Lysergic acid diethylamide and serotonin: a comparison of effects on serotonergic neurons and neurons receiving a serotonergic input, *J. Pharmacol. exp. Ther.*, 188 (1974) 688–699.
17 Heller, E., Kaczmarek, L. K., Hunkapiller, M. W., Hood, L. E. and Strumwasser, F., Purification and primary structure of two neuroactive peptides that cause bag cell afterdischarge and egg laying in *Aplysia*, *Proc. nat. Acad. Sci. U.S.A.*, 77 (1980) 2328–2332.
18 Heym, J., Trulson, M. E. and Jacobs, B. L., Effects of adrenergic drugs on raphe unit activity in freely moving cats, *Europ. J. Pharmacol.*, 74 (1981) 117–125.
19 Horn, J. P. and McAfee, D. A., Alpha-adrenergic inhibition of calcium-dependent potentials in rat sympathetic neurones, *J. Physiol. (Lond.)*, 301 (1980) 191–204.
20 Hubbard, J. I., Llinás, R. and Quastal, D. M. J., *Electrophysiological Analysis of Synaptic Transmission*, Williams and Wilkins, Baltimore, MD, 1969.
21 Katz, A. M., Tada, M. and Kirchberger, M. A., Control of calcium transport in the myocardium by the cyclic AMP-protein kinase system. In G. I. Drummon, P. Greengard and G. A. Robinson (Eds.), *Advances in Cyclic Nucleotide Research, Vol. 5*, Raven Press, New York 1975, pp. 453–472.
22 Kupfermann, I. and Kandel, E. R., Electrophysiological properties and functional interconnections of two symmetrical neurosecretory clusters (bag cells) in abdominal ganglion of *Aplysia*, *J. Neurophysiol.*, 33 (1970) 865–876.
23 Marwaha, J. and Aghajanian, G. K., Relative potencies of alpha-1 and alpha-2 antagonists in the locus coeruleus, dorsal raphe and dorsal lateral geniculate nuclei: an electrophysiological study, *J. Pharmacol. exp. Ther.*, 222 (1982) 287–293.
24 Menkes, D. B., Baraban, J. M. and Aghajanian, G. K., Prazosin selectively antagonizes neuronal responses mediated by α_1-adrenoceptors in brain, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 317 (1981) 273–275.
25 Mosko, S. B. and Jacobs, B. L., Recording of dorsal raphe unit activity in vitro, *Neurosci. Lett.*, 2 (1976) 195–200.
26 Reuter, H. and Scholz, H., The regulation of the Ca conductance of cardiac muscle by adrenaline, *J. Physiol. (Lond.)*, 264 (1977) 49–62.
27 Salmoiraghi, G. and Weight, F., Micromethods in neuropharmacology: an approach to the study of anesthesia, *Anesthesiology*, 28 (1967) 54–64.
28 Steinbusch, H. W. M., Distribution of serotonin-immunoreactivity in the central nervous system — Cell bodies and terminals, *Neuroscience*, 6 (1981) 557–618.
29 Steinbusch, H. W. M., Nieuwenhuys, R., Verhofstad, A. A. J. and Van der Kooy, D., The nucleus raphe dorsalis of the rat and its projection upon the caudatoputamen: a combined cytoarchitectonic, immunohistochemical and retrograde transport study, *J. Physiol. (Paris)*, 77 (1981) 157–174.
30 Svensson, T. H., Bunny, B. S. and Aghajanian, G. K., Inhibition of both noradrenergic and serotonergic neurons in

brain by the α-adrenergic agonist clonidine, *Brain Research*, 92 (1975) 291–306.

31 Trulson, M. E., Howell, G. A., Brandstetter, J. W., Frederickson, M. H. and Frederickson, C. J., In vitro recording of raphe unit activity: evidence for endogenous rhythms in presumed serotonergic neurons, *Life Sci.*, 31 (1982) 785–790.

32 Trulson, M. E. and Trulson, V. M., Chloral hydrate anesthesia alters the responsiveness of dorsal raphe neurons to psychoactive drugs, *Soc. Neurosci. Abstr.*, 8 (1982) 897.

33 Tsien, R. W. and Siegelbaum, S., Excitable tissues: the heart. In T. E. Andreoli, J. F. Hoffman, and D. D. Fanestil (Eds.), *Physiology of Membrane Disorders*, Plenum, New York, 1978, pp. 517–538.

34 VanderMaelen, C. P. and Aghajanian, G. K., Noradrenergic activation of serotonergic dorsal raphe neurons recorded in vitro, *Soc. Neurosci. Abstr.*, 8 (1982) 482.

35 Wang, R. Y. and Aghajanian, G. K., Antidromically identified serotonergic neurons in the rat midbrain raphe: evidence for collateral inhibition, *Brain Research*, 132 (1977) 186–193.

36 Wang, R. Y. and Aghajanian, G. K., Collateral inhibition of serotonergic neurones in the rat dorsal raphe nucleus: pharmacological evidence, *Neuropharmacology*, 17 (1978) 819–825.