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LSD Has High Efficacy Relative to Serotonin in Enhancing the Cationic Current I_h: Intracellular Studies in Rat Facial Motoneurons

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ABSTRACT The effects of LSD (d-lysergic acid diethylamide) on rat facial motoneurons were compared to those of 5-hydroxytryptamine (5-HT) in brain slices by means of current clamp and single-electrode voltage-clamp recordings. As previously reported, 5-HT, in part by decreasing a resting potassium conductance, produced a reversible depolarization (~5 mV), an increase in input resistance, and an enhancement in electrical excitability. LSD also produced an increase in electrical excitability, although with a much slower onset and longer duration. However, in contrast to 5-HT, LSD produced only a slight depolarization (1–2 mV). Moreover, in the presence of LSD the depolarizing effect of 5-HT was markedly attenuated. The 5-HT_2/5-HT_{1C} agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) produced effects intermediate between LSD and 5-HT. The LSD-induced increase in electrical excitability was completely reversed by spiperone, a 5-HT_2/5-HT_{1A} antagonist, and by ritanserin, a 5-HT_2/5-HT_{1C} antagonist; the effects of 5-HT were also reduced by these 2 antagonists, but complete blockade did not occur at the concentrations and durations tested. Surprisingly, LSD was found to enhance the hyperpolarization-activated nonspecific cation current I_h to a greater extent than did 5-HT; this enhancement was blocked by both spiperone and ritanserin. These results indicate that, despite having low efficacy relative to 5-HT in decreasing resting potassium conductance, LSD has high efficacy in enhancing the I_h current in rat facial motoneurons; possible mechanisms for this difference are discussed. © 1993 Wiley-Liss, Inc.

INTRODUCTION

There has been much interest in the role of the serotonergic system in the mechanism of action of LSD (d-lysergic acid diethylamide) and other psychedelic hallucinogenic drugs. A good correlation exists in rank order of affinity of hallucinogens for 5-hydroxytryptamine$_2$ (5-HT_2) receptors and hallucinogenic potency in humans (Glennon et al., 1984; Titeler et al., 1988). There has, however, been some debate on whether the hallucinogens act as agonists, partial agonists (Glennon, 1990; Sanders-Bush et al., 1988; Sheldon and Aghajanian, 1990), or antagonists (Pierce and Peroutka, 1990) at 5-HT_2 receptors. The discovery of the structural and functional similarities between the 5-HT_2 and 5-HT_{1C} receptors (see Hartig, 1989) has also led to studies examining the effect of the hallucinogens at 5-HT_{1C} sites. Recent data from studies in the choroid plexus showed that the hallucinogens act as partial agonists at 5-HT_{1C} receptors, mediating an activation of phosphoinositide turnover (Burris et al., 1991; Sanders-Bush and Breeding, 1991).

The facial motor nucleus receives a dense serotonergic innervation (Aghajanian and McCall, 1980) and has a high density of 5-HT_2 receptor binding sites and mRNA expression (Mengod et al., 1990; Pazos et al., 1985). 5-HT has been shown to induce a slow inward current and an increase in input resistance in rat facial motoneurons (FMNs) both in vivo (Rasmussen and Aghajanian, 1990; VanderMaelen and Aghajanian, 1980, 1982) and in vitro (Aghajanian and Rasmussen, 1989; Larkman et al., 1989; Rasmussen and Aghajanian, 1990). The 5-HT-induced inward current in FMNs has been shown to be mediated by G proteins and to be

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negatively modulated by protein kinase C (Aghajanian, 1990). In extracellular studies, the hallucinogens LSD, psilocin, and mescaline have been found to potentiate the excitatory response of rat FMNs to serotonin (McCall and Aghajanian, 1980). Intracellular studies in a brain slice preparation of rat facial motor nucleus have shown that the phenalkylamine hallucinogen, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), increases the electrical excitability of FMNs (Rasmussen and Aghajanian, 1990).

A previous study showed that FMNs have a hyperpolarization-activated cationic current (I_h) (Aghajanian and Rasmussen, 1989). Recently 5-HT has been shown to enhance the $I_h$current in several different regions of the mammalian brain (Bobker and Williams, 1989; McCormick and Pape, 1990; Nedergaard et al., 1991), including the facial motor nucleus (J.S. Kelly, personal communication). The present study compared the effects of the hallucinogens LSD and DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane] with 5-HT on FMNs in the in vitro brain slice preparation with respect to membrane potential, electrical excitability, and the I_h current.

MATERIALS AND METHODS

Tissue preparation

Brain slices were prepared using the sucrose method (Aghajanian and Rasmussen, 1989), which preserves the viability of FMNs in brain slices from adult rats. The normal artificial cerebrospinal fluid (ACSF) consited of (in mM): NaCl, 126; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 2; $NaHCO_3$, 26; NaH_2PO_4, 1.25; d-glucose, 10. During tissue preparation (blocking, slicing, and recovery) a modified ACSF was used in which equiosmolar sucrose (252 mM) was substituted for NaCl (126 mM).

Young adult male Sprague-Dawley rats (110–150 g; Charles River) were anaesthetized (chloral hydrate, 400 mg/kg i.p.) and perfused intracardially with 50 ml of cold (~4°C) sucrose-ACSF. Following decapitation, the brain was removed rapidly and placed in cold sucrose-ACSF. The brain was trimmed to yield a small block of brainstem containing the facial motor nucleus. Coronal slices (500 μm) were cut in cold sucrose-ACSF with a vibrating-knife microtome (Vibraslice, WPI). Slices were cut with the anterior face of the tissue block facing up. The ascending and exiting facial nerves, which are located just anterior to the facial motor nucleus, were used as surface landmarks for selecting the slice to be taken for study. Slices were transferred to the stage of a gas–liquid interface type of brain slice chamber. The stage of the chamber, over which humidified 95% O_2/5% CO_2 flowed, was initially kept at room temperature (~23°C); after placement of the slice into the chamber, the temperature was gradually raised over 30 min to a final level of 31–32°C. Slices were maintained in sucrose-ACSF for approximately 1 hour before being switched to normal ACSF for the remainder of the experiment.

Intracellular techniques

Intracellular recording, current injection, and single-electrode voltage clamping were conducted using an Axoclamp-2 (Axon Instruments, Burlingame, CA) as previously described (Aghajanian and Rasmussen, 1989). Stubby electrodes (~8 mm, shank to tip), with relatively low capacitance and resistance (25–45 MΩ), were pulled with a Brown-Flaming Micropipette Puller (Sutter Instruments, San Francisco, CA) using fused-filament capillary tubing (1.5 mm O.D., WPI); electrodes were filled with 2 M KCl. Electrodes prepared in this manner had rapid settling times (50–75 μs), allowing voltage-clamp switching frequencies of 4–6 Hz and a loop gain of 10 nA/mV (30% duty cycle). Phase lag was used to avoid oscillations; false clamping was avoided by utilizing optimal capacitance neutralization and by selecting a switching frequency that allowed full settling to a horizontal base line, as verified by monitoring headstage input voltage continuously (Finkel and Redman, 1984).

Cells were impaled by inducing short pulses of high-frequency oscillation (Axoclamp Buzz Duration control) while advancing through the slice in 4 micron steps. Resting potentials of FMNs ranged from −68 to −77 mV, and input resistances (assessed by passing 1 nA hyperpolarizing pulses through the bridge circuit of the Axoclamp-2) were between 7 and 20 MΩ; this is in accord with previous reports (Aghajanian and Rasmussen, 1989; Rasmussen and Aghajanian, 1990). Electrical excitability was measured by injecting cells with a constant-current depolarizing pulse (400 ms) adjusted to a level which elicited 1 or 2 spikes. In the presence of the drug solutions, a change in the electrical excitability of the facial motoneuron was observed as an increase or decrease in the number of spikes/pulse. Reversal potentials for the 5-HT-induced currents were ascertained by plotting current-voltage plots (I/V curves) before and after 5-HT application using slow ramps (4 mV/s) to allow for steady-state conditions to be attained. The ramps were generated using pClamp software (Axon Instruments) on an IBM-AT clone. The acquired data were transferred to a Mac IISi and the I/V curves were plotted using the Axograph software (Axon Instruments).

Drugs were bath applied by gravity feed and solutions were switched by way of 3-way stopcocks (Baxter Healthcare, CA). For purposes of efficacy comparisons, drugs were given at near-maximal concentrations based on previous studies for 5-HT (Aghajanian and Rasmussen, 1989; Rasmussen and Aghajanian, 1990) and preliminary experiments for DOI and LSD.

To determine the reversal potential for I_h, the slices were bathed in ACSF containing TTX (1 μM) to block

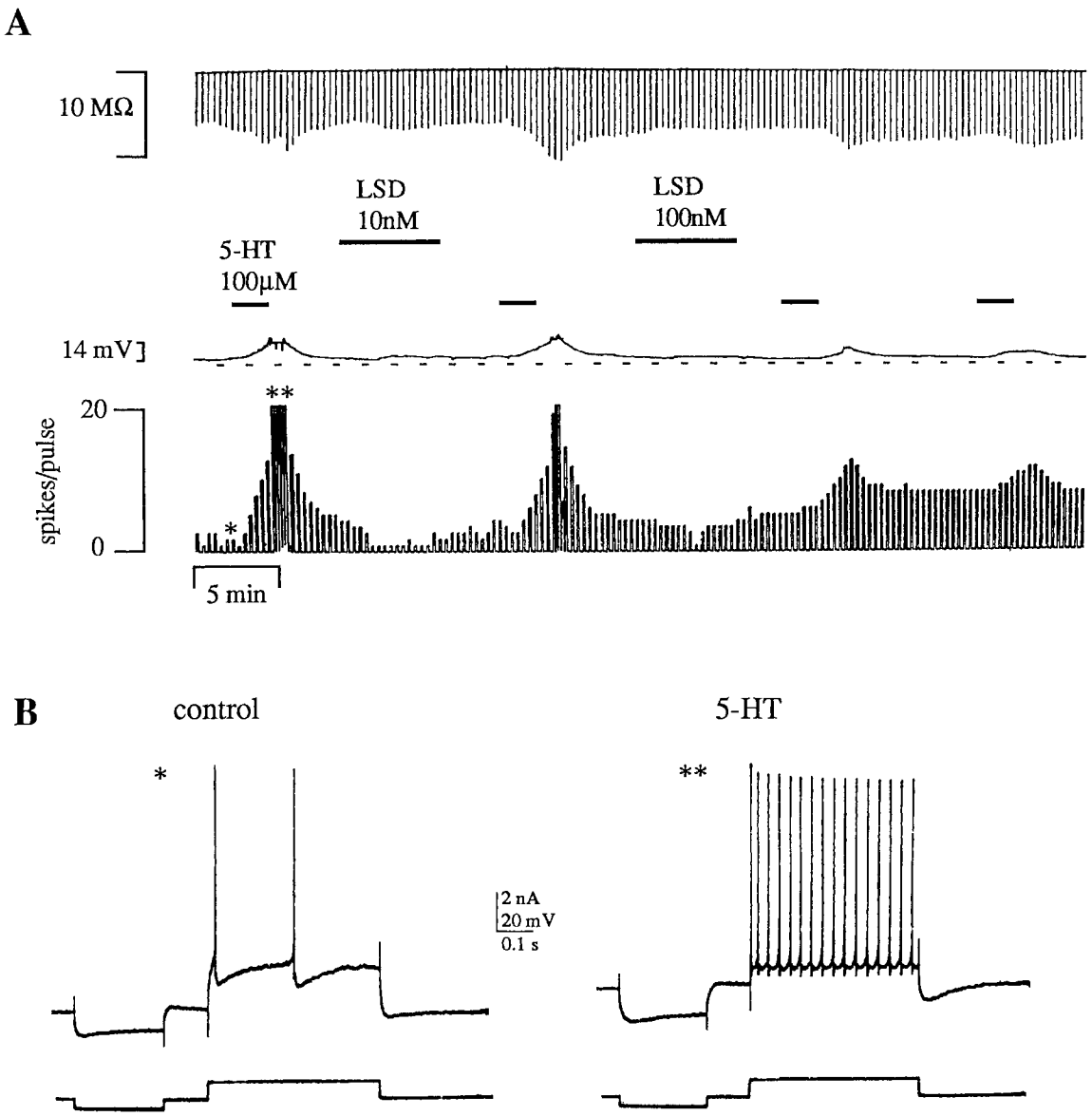

Fig. 1. A: Effects of bath application of 5-HT and LSD on input resistance (upper trace), membrane potential (middle trace), and electrical excitability (lower trace) recorded simultaneously in a rat facial motoneuron from an in vitro brain slice preparation. Bath application of 5-HT (100 μM) produced an increase in the apparent input resistance, a depolarization (14 mV), and an increase in electrical excitability of the cell; note that the ratemeter trace goes off scale. Application of LSD (10 nM) produced little change in the apparent input resistance or membrane potential; however, there was a clear increase in electrical excitability, which had a slow onset of action. Interestingly, LSD at this dose slightly suppressed the response of the cell to 5-HT. A higher dose of LSD (100 nM) again produced little change in the apparent input resistance or membrane potential. There was, however, a further increase in the electrical excitability, which also had a slow onset of action and very long duration (>2 hr, not shown). At this dose there was a greater suppression of the response of the cell to 5-HT. Electrical excitability was measured by injecting the cell with a depolarizing pulse (+1.5 nA, 400 ms duration) which elicited 1 or 2 spikes under control conditions. Periods of drug application are indicated by horizontal bars. B: Oscilloscope traces showing the effect of 5-HT on the electrical excitability of the facial motoneuron shown in A. The spikes were evoked by a depolarizing pulse (+1.5 nA), and the cell was otherwise silent (resting potential, −72 mV). The left-hand panel shows that under control conditions 2 spikes were elicited to the depolarizing pulse (single asterisk denotes time point that sweep was taken in A). In the presence of 5-HT (100 μM; in the right-hand panel) the same pulse elicited 16 spikes (double asterisk in A), thus indicating that 5-HT increases the electrical excitability of the cell. Also, note the increase in input resistance (i.e., greater voltage deflection in response to the hyperpolarizing pulse) following 5-HT.

fast sodium currents. Calcium currents were blocked by lowering the Ca^{2+} concentration to 0.5 mM and adding cobalt ($CoCl_2$, 2 mM) to the ACSF; to prevent precipitation of the cobalt, Tris hydrochloride was substituted for NaH_2PO_4. Under these conditions, the membrane potential of the cell was held at −70 mV, briefly

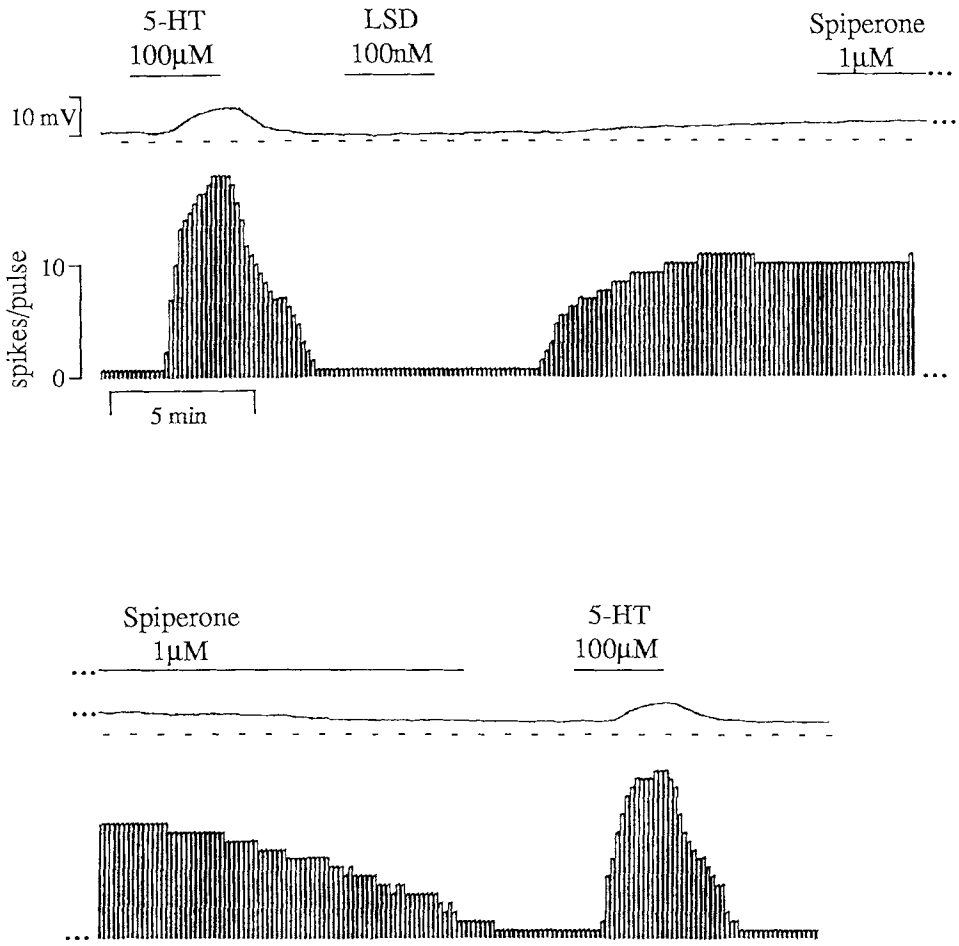

Fig. 2. Effects of bath application of 5-HT, LSD, and the 5-HT_2/5-HT_{1A} antagonist, spiperone, on membrane potential and excitability recorded simultaneously in a rat facial motoneuron from an in vitro brain slice preparation. The pair of traces in the bottom panel of the figure are a direct continuation in time of the pair of traces in the top panel. Bath application of 5-HT (100 μM) produced a depolarization (8 mV) and increased the number of spikes in response to a constant depolarizing pulse. LSD (100 nM) produced a slight depolarization and increased the electrical excitability of the cell; this effect had a slow onset and a very long duration. Spiperone (1 μM, bath applied for 20 min) reversed the LSD-induced increase in electrical excitability of the cell. A second application of 5-HT produced a smaller depolarization (5 mV) and a smaller increase in the number of spikes elicited by the constant current pulse. Electrical excitability was measured by injecting the cell with a depolarizing pulse (+1.2 nA, 400 ms duration), which elicited 1 spike under control conditions. Periods of drug application are indicated by horizontal bars.

stepped to −120 mV to activate I_h, and then stepped back to various membrane potentials (−70 to 0 mV).

Chemicals

Serotonin creatinine sulphate (5-HT), tetrodotoxin (TTX), and Tris hydrochloride were from Sigma. LSD was from NIDA. DOI, ritanserin, spiperone, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), and CGS 12066B maleate were from Research Biochemicals Incorporated (RBI). Inorganic salts and sucrose were of analytic chemical grade.

RESULTS

In agreement with previous findings, both in vivo (Rasmussen and Aghajanian, 1990; VanderMaelen and Aghajanian, 1980, 1982) and in vitro (Aghajanian and Rasmussen, 1989; Larkman et al., 1989; Rasmussen and Aghajanian, 1990), the bath application of 5-HT (100 μM) to FMNs in the brain slice preparation caused a moderate depolarization (5.1 ± 0.5 mV; mean ± S.E.M., n = 17), an increase in input resistance, and an increase in electrical excitability (from ~1 spike/pulse to 16.9 ± 1.6 spikes/pulse; n = 17; Fig. 1). LSD also increased the electrical excitability of FMNs (Fig. 1), although in contrast to 5-HT this effect had a slow onset of action, an extremely long duration, and was accompanied by only a very small depolarization (1–2 mV). In addition, LSD initially produced a small, transient hyperpolarization (1–2 mV). Concomitantly, LSD dose-dependently (10 nM to 1 μM) suppressed both the maximal depolarization and the increase in electrical excitability produced by 5-HT (Fig. 1). DOI (10 μM) also

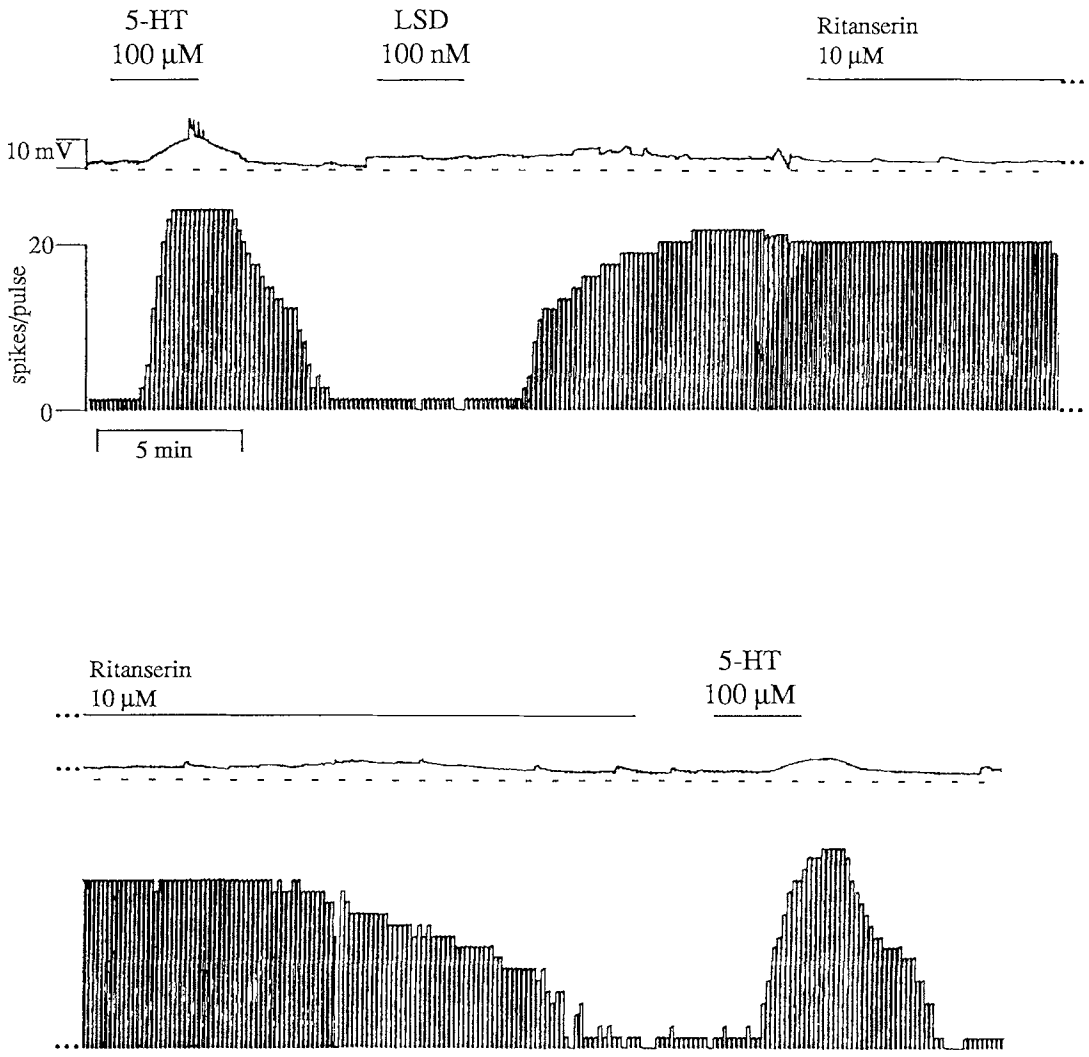

Fig. 3. Effects of bath application of 5-HT, LSD, and the 5-HT_2/5-HT_{1C} antagonist, ritanserin, on membrane potential and excitability recorded simultaneously in a rat facial motoneuron from an in vitro brain slice preparation. The pair of traces in the bottom panel of the figure are a direct continuation of the pair of traces in the top panel. Bath application of 5-HT (100 μM) produced a depolarization and increased the number of spikes in response to a constant depolarizing pulse. Note that the trace went off scale and that there was a small artifactual DC shift just after the cell recovered from the application of 5-HT. Application of LSD (100 nM) produced a slight depolarization and increased the electrical excitability of the cell; this effect had a slow onset and long duration. Bath application of ritanserin (10 μM, 30 min) suppressed the LSD-induced increase in excitability of the cell. A second application of 5-HT produced a smaller depolarization (5 mV) and a smaller increase in the number of spikes elicited to the constant current pulse. Electrical excitability was measured by injecting the cell with a depolarizing pulse (+1.2 nA, 400 ms duration), which elicited 1 or 2 spikes under control conditions. Periods of drug application are indicated by horizontal bars.

increased the electrical excitability of FMNs and, as in the case of LSD, this effect had a slow onset of action and a very long duration (>2 hr), and was accompanied with a small to moderate depolarization (2–3 mV; n = 8; not shown). DOI, like LSD, reduced the maximal effects of 5-HT on the FMNs.

The 5-HT_{1A} agonist 8-OH-DPAT (1–10 μM; Middlemiss and Fozard, 1983) had no effect on the electrical excitability of FMNs and did not alter the response of FMNs to 5-HT (n = 7) (not shown). The selective 5-HT_{1B} agonist, CGS 12066B maleate (100 nM to 1 μM; Neale et al., 1987), also failed to produce any effect on FMNs; it too did not alter the response of the motoneurons to 5-HT (n = 6) (not shown).

The increase in electrical excitability of FMNs produced by LSD (100 nM) could be completely reversed by administration of both the 5-HT_2/5-HT_{1A} antagonist spiperone (1 μM; 15–30 min; n = 4; Fig. 2) and the 5-HT_2/5-HT_{1C} antagonist ritanserin (10 μM; 20–30 min; n = 3; Fig. 3). Both antagonists reversed the effects of LSD at a time when LSD would have been exerting a maximal response on the FMNs, since, in the absence of antagonists, the LSD-induced increase in electrical excitability did not recover during a recording

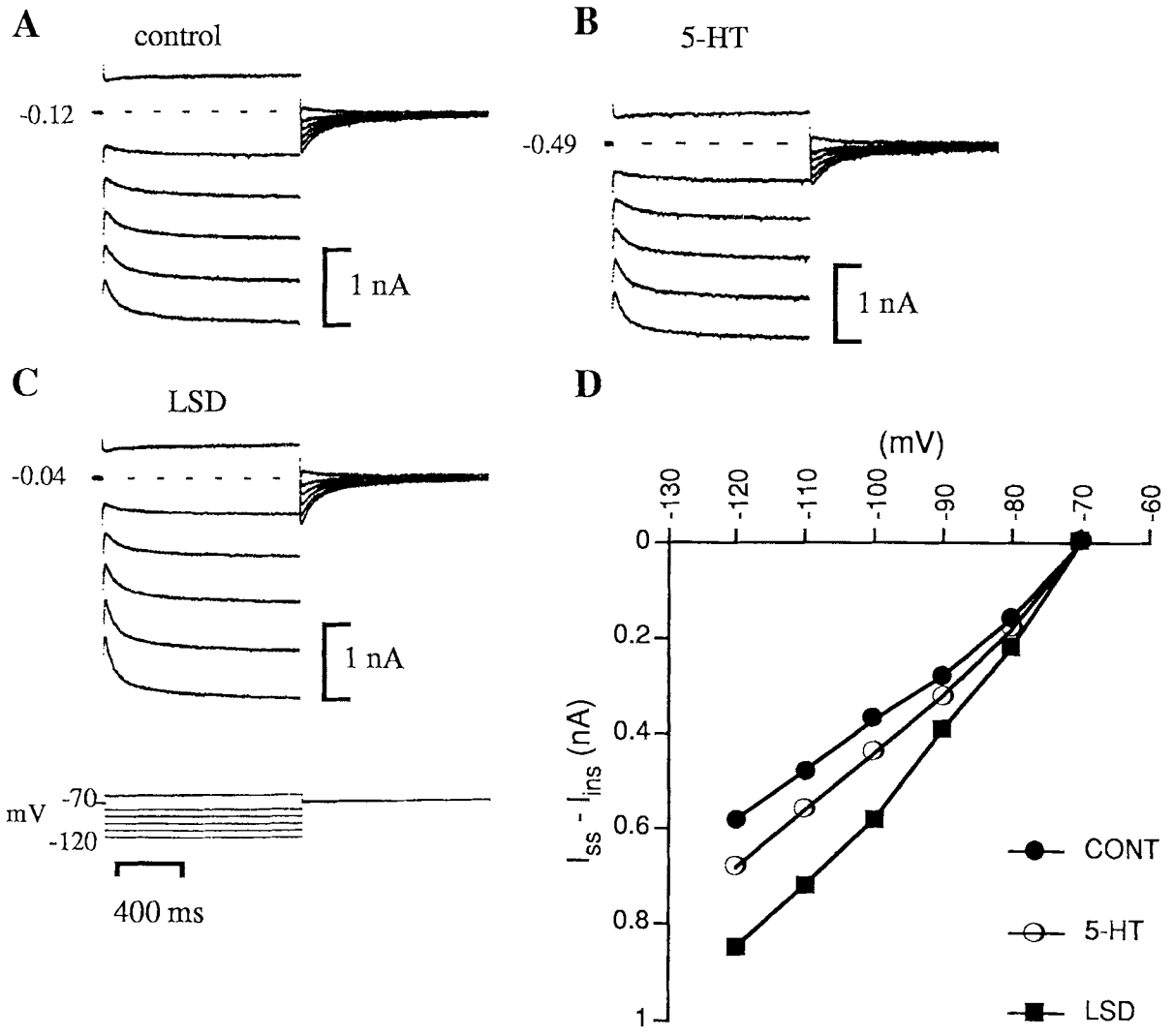

Fig. 4. Effects of bath application of 5-HT and LSD on the hyperpolarization-activated cation current (I_h) in facial motoneurons. A–C: Oscilloscope traces showing intracellular voltage-clamp recordings from a facial motoneuron. A shows a progressive increase in the size of the hyperpolarization-activated cation current (I_h) in response to increased hyperpolarizing steps from −70 to −120 mV. B shows the enhancement of I_h in the presence of 5-HT (100 μM). C shows that LSD (100 nM) produces a greater increase in I_h than 5-HT. D: A current-voltage plot obtained from A–C showing the difference between steady-state and instantaneous currents in response to voltage clamp commands in 10 mV steps from a holding potential of −70 to −120 mV. Note that LSD produced a greater increase in I_h at all voltage steps.

period of up to 120 min (n = 4; not shown). The small depolarization and sustained increase in electrical excitability produced by DOI (10 μM) were also totally reversed by bath application of spiperone (1 μM; 15–30 min; n = 3) and ritanserin (10 μM; 20–45 min; n = 3; not shown). In contrast, spiperone only partially suppressed the 5-HT-induced increase in electrical excitability and depolarization (32 ± 5% and 38 ± 9%, respectively; Fig. 2, n = 5). Ritanserin (10 μM) also produced only a very small decrease in the increased electrical excitability produced by 5-HT and reduced the depolarization by 45 ± 8% (Fig. 3, n = 3).

As in several other brain regions (Bobker and Williams, 1989; McCormick and Pape, 1990; Nedergaard et al., 1991), 5-HT (100 μM) was found to enhance I_h in FMNs. Surprisingly, in view of its low efficacy in depolarizing FMNs, LSD (100 nM) produced a substantially greater increase in I_h than did 5-HT (40% compared with 20%; Figs. 4 and 5). The effect of LSD on I_h, like that on the electrical excitability, had a slow onset of action and a very long duration. DOI (10 μM) enhanced I_h to a degree intermediate between 5-HT and LSD (Fig. 5). As both spiperone and ritanserin had been found to block the increase in electrical excitability on FMNs produced by LSD, the effects of these compounds were examined on the enhancement of I_h produced by LSD. The LSD-induced enhancement of I_h was largely reversed by bath applications of the 5-HT_2/

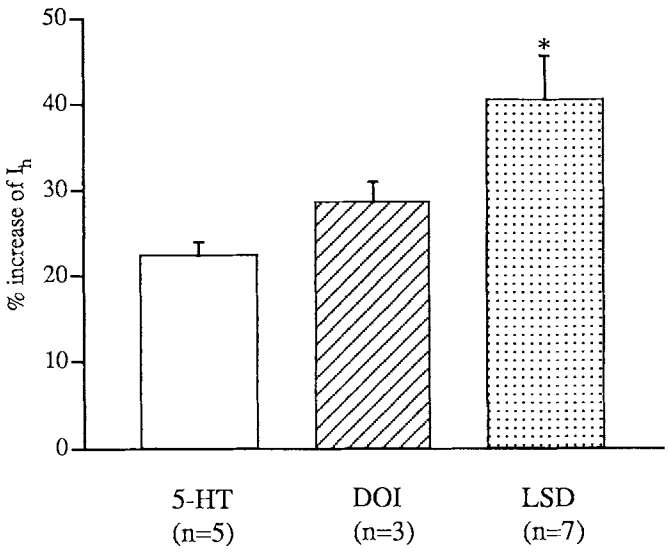

Fig. 5. The bar chart shows the effect of 5-HT (100 μM), DOI (10 μM), and LSD (100 nM) on the hyperpolarization-activated cation current (I_h) in rat facial motoneurons. All the compounds enhanced I_h and, interestingly LSD was found to produce the greatest increase in I_h. Values are mean ± S.E.M. *$P < 0.01$ as compared to 5-HT using the Scheffe F-test.

5-HT_{1A} antagonist, spiperone, (1 μM; ~10 min; n = 2; Fig. 6) and by the 5-HT_2/5-HT_{1C} antagonist, ritanserin (10 μM; ~15 min; n = 3; Fig. 7).

It has been suggested that the depolarization of FMNs by 5-HT is mediated by a decrease in resting conductance to potassium (VanderMaelen and Aghajanian, 1982). If the effects of 5-HT were solely mediated by the closing of potassium channels, the expected reversal potential should be close to the reversal potential for potassium (−90 mV). In practice, however, it has been very difficult to demonstrate a reversal potential for the 5-HT-induced inward current within this voltage range (Larkman et al., 1989; G.K. Aghajanian unpublished observations). Since 5-HT enhances I_h in FMNs, it is possible that I_h could have a contaminating effect on the determination of the reversal potential for the potassium component of the 5-HT-induced inward current. To examine this issue more closely, the reversal potential for I_h was determined by bathing the slice in ACSF containing $CoCl_2$ (2 mM) and reduced Ca^{2+} (0.5 mM) to suppress voltage-activated Ca^{2+} currents and TTX (1 μM) to prevent Na^+ spikes. The membrane potential of the cell was held at −70 mV, briefly stepped to −120 mV to activate I_h, and then stepped back to various membrane potentials (−70 mV to 0 mV). The reversal potential for the resulting tail currents was approximately −30 mV (Fig. 8), thus having the characteristics of a mixed Na^+/K^+ cationic channel (see Aghajanian and Rasmussen, 1989). This agrees with values obtained for the reversal potential of I_h in other areas (e.g., McCormick and Pape, 1990; Nedergaard et al., 1991; Uchimura et al., 1990).

As I_h would be strongly activated at the potassium reversal potential (approximately −90 mV, assuming an intracellular K^+ concentration of 150 mM), an increase in I_h induced by serotonin would shift the apparent reversal potential of the potassium component of the 5-HT-induced inward current away from E_k. Accordingly, we found that when I_h was suppressed by CsCl (see Aghajanian and Rasmussen, 1989), the reversal potential for the 5-HT-induced current was approximately −95 mV (n = 2; Fig. 9), consistent with the suggestion that this current is predominantly mediated by a closing of K^+ channels. Also note that the magnitude of the inward current was reduced following CsCl, possibly indicating the blockade of a non-inactivating component of I_h at resting potential. Following washout of CsCl, the I/V curves reverted to control state in that they did not cross in the voltage range tested (−70 to −130 mV; not shown). As the LSD-induced depolarization was very small, the effect of CsCl on the reveral potential was not examined.

DISCUSSION

The results of this study show that 5-HT exerts 2 different effects on FMNs: 1) a decrease in the resting potassium conductance; 2) an enhancement of the nonspecific cationic current I_h; LSD and DOI have low intrinsic activity relative to 5-HT on the decrease in potassium conductance and therefore act as partial agonists. This is in agreement with studies looking at the effects of these compounds on phosphoinositide hydrolysis (Barker et al., 1991; Sanders-Bush et al., 1988). In the latter studies, 5-HT was found to cause the greatest increase in phosphoinositide hydrolysis, whereas 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB), DOM and LSD acted as partial agonists, with LSD having the least intrinsic activity. In the present study, in contrast to its low efficacy in decreasing resting potassium conductance, LSD produced a greater enhancement of the cationic current I_h than did 5-HT, indicating that it has higher efficacy than 5-HT with respect to I_h in rat FMNs. We believe that this is the first example of a 5-HT_2 response in brain in which LSD has been found to have greater efficacy than 5-HT (Pierce and Peroutka, 1990; Sanders-Bush et al., 1988).

The increase in electrical excitability of FMNs produced by LSD was reversed by application of both the 5-$HT_2$15-HT_{1A} antagonist, spiperone, and the 5-HT_2/5-HT_{1C} antagonist, ritanserin. As both these compounds appear to share antagonistic actions only at the 5-HT_2 receptor, this would suggest that the increase in electrical excitability produced by LSD is mediated by 5-HT_2 receptors. Extended application of both ritanserin and spiperone at the concentrations used, however, only partially reduced the response of FMNs to 5-HT. Although ritanserin given in vivo rapidly blocks the excitatory effects of 5-HT (Rasmussen and Aghajanian, 1990), the present finding is in agreement with other in

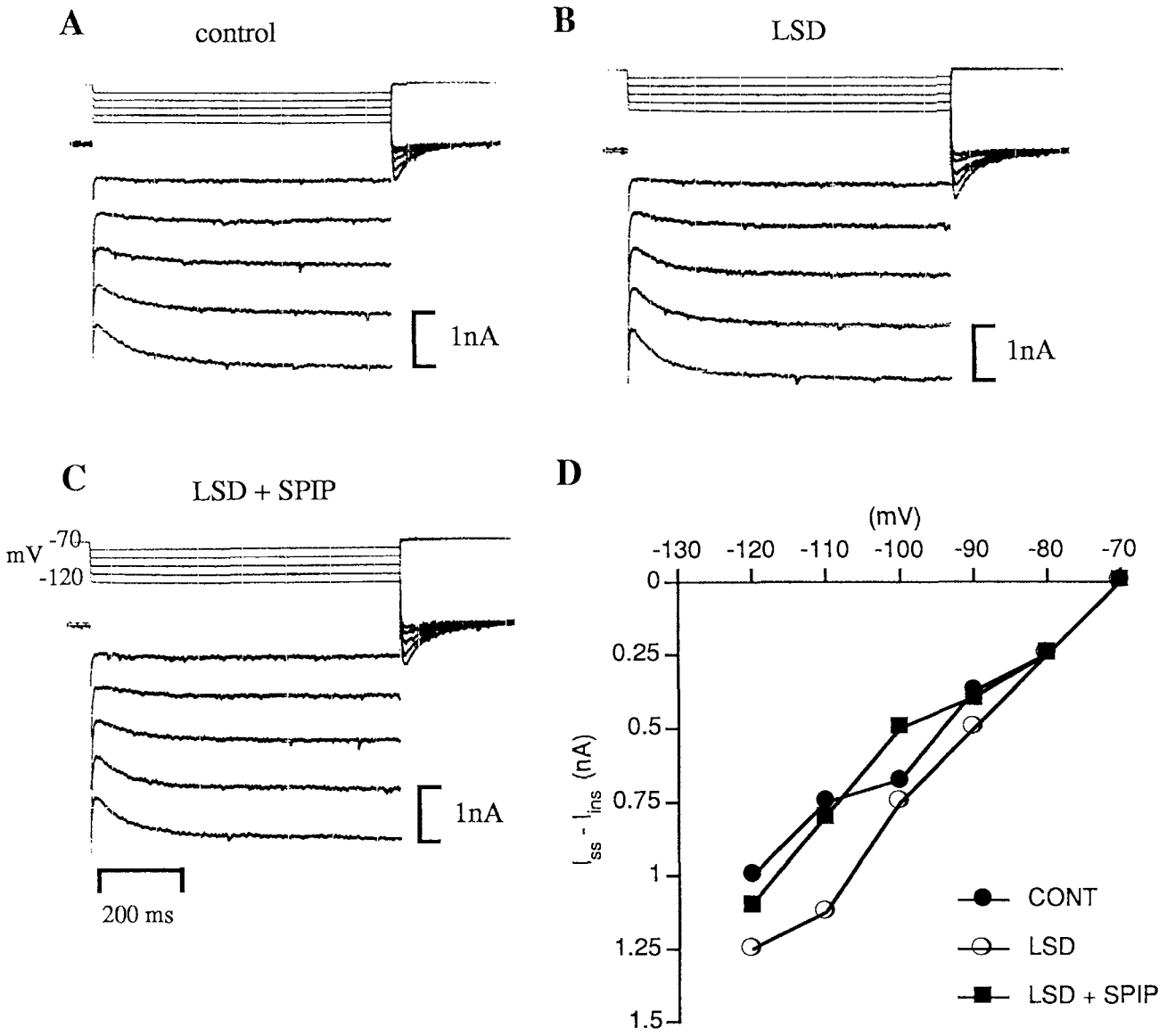

Fig. 6. Effect of bath application of spiperone on the LSD-induced increase in I_h. A–C: Oscilloscope traces showing intracellular voltage-clamp recordings from a facial motoneuron. A shows a progressive increase in the size of hyperpolarization-activated cation current (I_h) in response to increasing hyperpolarizing steps from −70 to −120 mV. B shows the increase of I_h in the presence of LSD (100 nM). C shows that the 5-HT_2/5-HT_{1A} antagonist, spiperone (1 μM), blocked the increase in I_h produced by LSD (100 nM). D: A current-voltage plot obtained from A–C showing the difference between steady-state and instantaneous currents in response to voltage-clamp commands in 10 mV steps from a holding potential of −70 to −120 mV.

vitro studies, which have shown that ritanserin has a very slow onset of action in slice preparations (Connell and Wallis, 1989; Rasmussen and Aghajanian, 1990). The slow onset of action of spiperone in FMN slices also agrees with a study in the in vitro preparation of neonatal rat spinal motoneurons (Connell and Wallis, 1988). Why these compounds take much longer to produce their inhibitory effects on 5-HT in vitro in comparison to in vivo remains unclear.

In agreeement with microiontophoretic studies in vivo (Rasmussen and Aghajanian, 1990), the 5-HT_{1A} agonist 8-OH-DPAT did not mimic the effects of 5-HT on FMNs in vitro, indicating that 5-HT_{1A} receptors do not mediate the effects of 5-HT in the FMNs. Although LSD is a potent agonist at 5-HT_{1A} autoreceptors, the lack of effect of 8-OH-DPAT on the FMNs makes it unlikely that the effects of LSD are mediated via 5-HT_{1A} receptors. Moreover, binding studies have shown that there are very few 5-HT_{1A} binding sites in the facial motor nucleus (Thor et al., 1992). LSD also binds to 5-HT_{1B} receptors, although with lower affinity than at 5-HT_{1A}, 5-HT_{1C}, and 5-HT_2 sites (Hoyer, 1988).

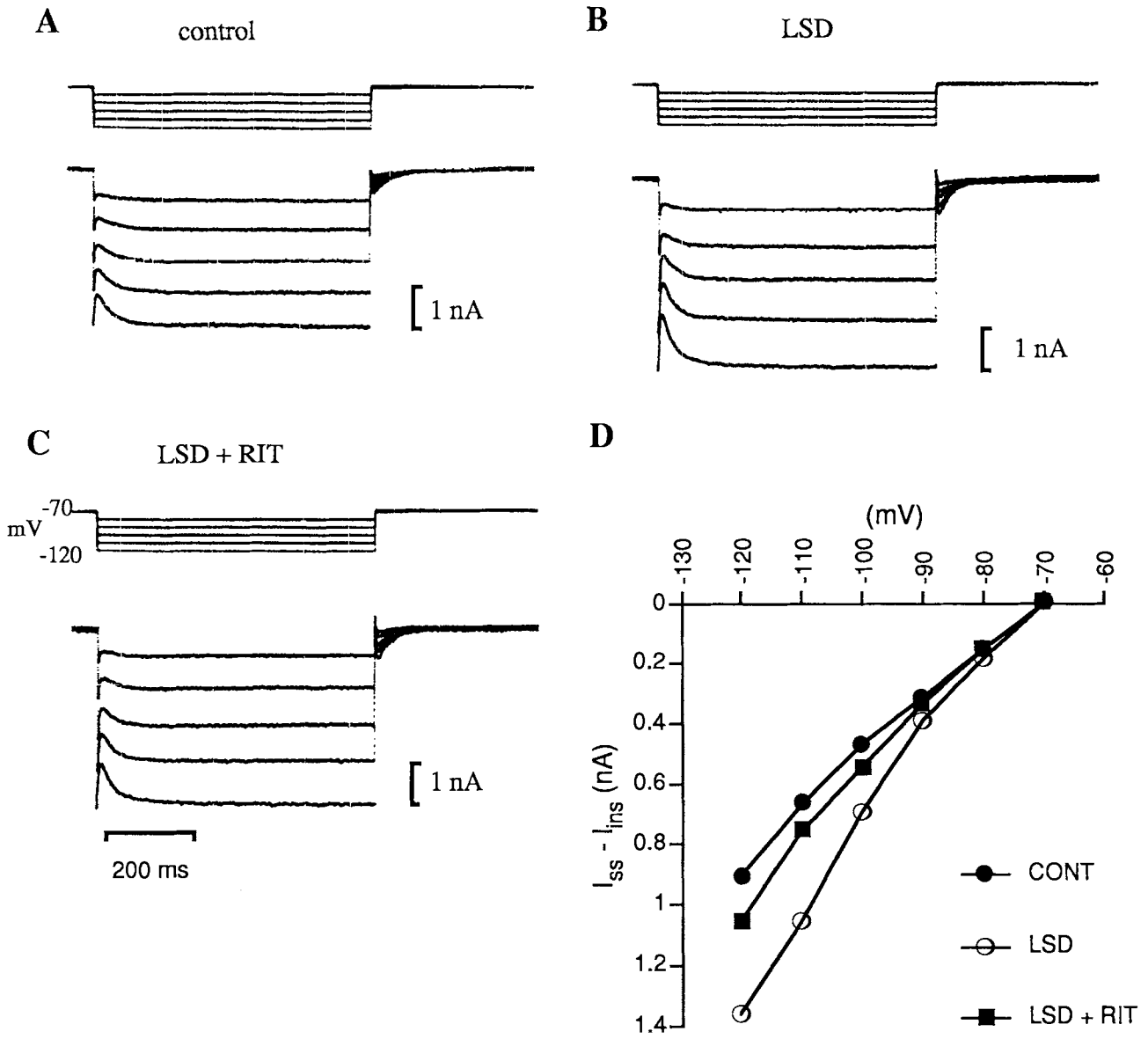

Fig. 7. Effect of bath application of ritanserin on the LSD-induced increase in I_h. A–C: Oscilloscope traces showing intracellular voltage-clamp recordings from a facial motoneuron. A shows a progressive increase in the size of hyperpolarization-activated cation current (I_h) in response to increasing hyperpolarizing steps from −70 to −120 mV. B shows the increase of I_h in the presence of LSD (100 nM). C shows that ritanserin (10 μM), a 5-HT_2/5-HT_{1C} antagonist, blocked the increase in I_h produced by LSD. D: A current-voltage plot obtained from A–C showing the difference between steady-state and instantaneous currents in response to voltage-clamp commands in 10 mV steps from a holding potential of −70 to −120 mV.

The 5-HT_{1B} agonist, CGS 12066B, was, however, found to be without effect on FMNs and did not alter the response to 5-HT. Thus, it seems unlikely that 5-HT_{1B} receptors are involved in mediating the effects of 5-HT or LSD. Although the facial motor nucleus has moderately dense 5-HT_{1B} binding, it has been suggested that these sites are associated with the terminals from corticoreticular and corticospinal inputs (Thor et al., 1992). Taken together, the results from the present study and that of Rasmussen and Aghajanian (1990) suggest that the effects of LSD and 5-HT on FMNs are mediated through 5-HT_2 receptors.

The phenalkylamine hallucinogen DOI produced effects similar to that of LSD on the excitability of the FMNs, although the depolarization was of a slightly greater magnitude. Moreover, DOI, like LSD, was able to suppress both the depolarization and increase in electrical excitability produced by 5-HT. These findings agree with a previous intracellular study which examined the effects of another phenalkylamine hallucino-

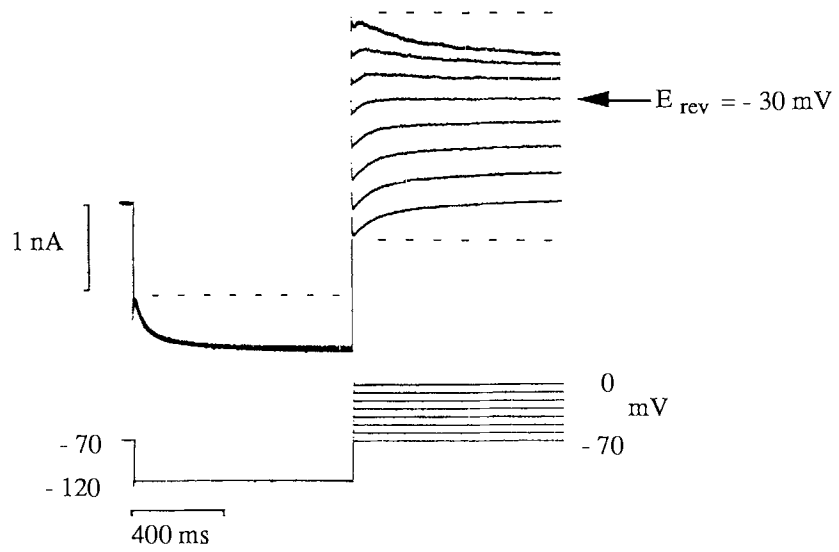

Fig. 8. Determination of the value for the reversal potential of I_h in a FMN under voltage clamp. To determine the reversal potential of I_h, the slice was bathed in ACSF containing cobalt (2 mM) and low Ca^{2+} (0.5 mM; to block voltage-activated Ca^{2+} currents) and TTX (1 μM; to block Na^+ spikes). The membrane potential of the cell was held at −70 mV, briefly stepped to −120 mV to activate I_h, and then stepped back to various membrane potentials (−70 to 0 mV). The reversal potential for the slow component of the resulting tail currents was approximately −30 mV, thus having the characteristics of a mixed Na^+/K^+ cationic channel.

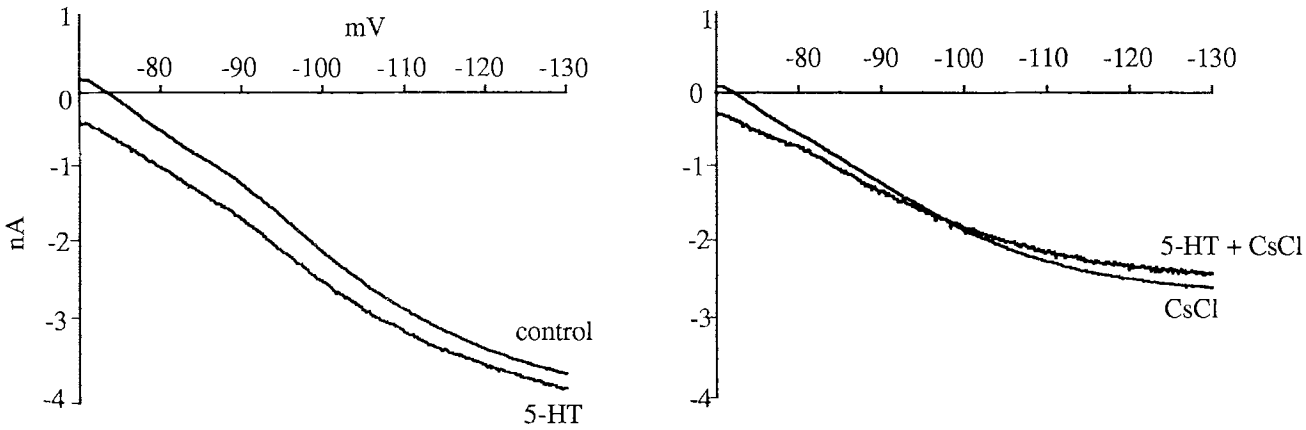

Fig. 9. Effect of external CsCl on the reversal potential of the 5-HT-induced inward current in facial motoneurons. The left panel shows that under control conditions the 5-HT response did not reverse in the voltage range tested (−70 to −130 mV), resulting in nearly parallel I/V curves. The right panel shows that in the same cell following application of external CsCl (2 mM), the 5-HT response reversed at −95 mV. Note also that the overall magnitude of the 5-HT-induced current was reduced. Following washout of CsCl, the I/V curves reverted to the control state in that they did not cross in the voltage range tested (−70 to −130 mV; not shown). All the I/V curves were obtained by applying 60 mV hyperpolarizing ramps at a rate of 4 mV/s.

gen, DOM, on FMNs in vitro (Rasmussen and Aghajanian, 1990) and an extracellular study looking at the effects of DOM on interneurons in the in vitro slice preparation of rat piriform cortex (Sheldon and Aghajanian, 1990). The increase in electrical excitability and depolarization produced by DOI in FMNs were blocked by extended application of ritanserin, a $5\text{-}HT_2/5\text{-}HT_{1C}$ antagonist, in accord with the in vitro study of the effects of DOM on FMNs (Rasmussen and Aghajanian, 1990). These effects were also blocked by prolonged application of the $5\text{-}HT_2/5\text{-}HT_{1A}$ antagonist, spiperone. These results suggest that the effects of DOI on the decrease in resting potassium conductance, like those of LSD and 5-HT, are mediated by $5\text{-}HT_2$ receptors.

The 5-HT-induced enhancement of I_h in adult FMNs in the brain slice preparation is in accord with studies in other brain regions that have shown 5-HT to increase I_h (Bobker and Williams, 1989; McCormick and Pape, 1990; Nedergaard et al., 1991; Takahashi and Berger, 1990). Interestingly, both LSD and DOI were found to produce a greater enhancement of I_h than did 5-HT (LSD > DOI > 5-HT). However, in contrast to 5-HT, the effects of DOI and LSD on I_h had a slow onset of action and a very long duration (>2 hr). The enhancement of

I_h produced by LSD was reversed by application of both the 5-HT_2/5-HT_{1A} antagonist, spiperone, and the 5-HT_2/5-HT_{1C} antagonist, ritanserin, suggesting that the increase in I_h produced by LSD is mediated by 5-HT_2 receptors.

The finding that, relative to 5-HT, LSD has high efficacy on I_h and low efficacy on the decrease in resting potassium conductance could be explained most simply by the existence of 2 different 5-HT_2 receptor subtypes which couple independently to the 2 different channels. This possibility requires that the agonists display differential intrinsic activities at the two 5-HT_2 receptor subtypes. A second possibility is that a single receptor subtype couples to the 2 different channels via 2 different G proteins (Ashkenazi et al., 1987). Thus, 5-HT_2 receptors may couple to potassium channels via one type of G protein and to I_h channels via another type of G protein, yielding 2 different receptor/G protein complexes which could confer differential intrinsic activity to 5-HT and LSD.

Regardless of the mechanism, the high efficacy of LSD relative to 5-HT on the I_h current in rat FMNs and the finding that this effect has an extremely long duration of action raises the interesting possibility that such an action of LSD elsewhere in brain could be involved in mediating its hallucinogenic properties. Neurons in several brain regions have been shown to have an I_h current which is enhanced by 5-HT (Bobker and Williams, 1989; McCormick and Pape, 1990; Nedergaard et al., 1991; Takahashi and Berger, 1990). It would be interesting to see if LSD produced a greater enhancement of I_h relative to 5-HT in other brain regions, especially those in which the effect on I_h may be mediated by a 5-HT_2 receptor.

In FMNs it has been difficult to demonstrate the reversal potential for the 5-HT-induced inward current (Larkman et al., 1989; G.K. Aghajanian, unpublished observations). Although the 5-HT-induced inward current is thought to be mediated through a decrease in resting membrane conductance to potassium (Aghajanian and Rasmussen, 1989; Larkman et al., 1989; VanderMaelen and Aghajanian, 1980, 1982), if this were the only action of 5-HT the reversal potential should be close to −90 mV, the expected reversal potential for potassium. In the present study, since 5-HT was found to enhance I_h in the FMNs, I_h would be strongly activated at the potassium reversal potential.

Thus, the 5-HT induced increase in I_h would mask the reversal of the potassium component of the 5-HT-induced inward current. After blockade of I_h with CsCl the reversal potential for the 5-HT current was found to be approximately −95 mV, suggesting that this current is due predominantly to a closure of K^+ channels.

This value for the reversal potential of 5-HT in the presence of CsCl is in accord with the extrapolated findings of Larkman et al. (1989).

In conclusion, the 5-HT-induced depolarization and increase in electrical excitability in FMNs appears to have 2 components: 1) a decreased resting potassium conductance and 2) an enhanced hyperpolarization-activated cationic current (I_h). When I_h was blocked by CsCl, the potassium component of the 5-HT-inward current reversed near the expected reversal potential for potassium. LSD had low efficacy on the decrease in potassium conductance and, therefore, acted as a potent partial agonist in relation to the depolarizing effect of 5-HT. In contrast, LSD was found to have high efficacy relative to 5-HT on I_h in rat FMNs. Alternative explanations for the difference in efficacy between 5-HT and LSD on these 2 currents are 1) that there are 2 subtypes of 5-HT_2 receptors having differential efficacy with respect to the 2 agonists or 2) that there is a single type of 5-HT_2 receptor whose agonist efficacy can be altered by its coupling to different G proteins. Further studies will be required to distinguish between these and other possible alternatives.

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