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Effects of the two antidepressant drugs mianserin and indalpine on the serotonergic system: Single-cell studies in the rat

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Abstract. Several antidepressant treatments enhance serotonergic neurotransmission. The present electrophysiological studies were undertaken to assess the effect of mianserin and indalpine, two antidepressant drugs with different pharmacological profiles, on serotonergic neurotransmission. In a first series of experiments, the responsiveness of hippocampal pyramidal neurons to microiontophoretic applications of serotonin (5-HT), norepinephrine (NE) and γ-aminobutyric acid (GABA) was assessed following mianserin, imipramine (5 mg/kg/day IP) or saline administration for 14 days. At 48 h after the last dose of mianserin, responsiveness to 5-HT was increased whereas that to NE and GABA was not modified. The degree of sensitization to 5-HT was the same as that produced by imipramine. Acute IV administration of mianserin (up to 10 mg/kg) did not decrease the firing rate of dorsal raphe 5-HT neurons. In a second series of experiments, long-term administration of indalpine (5 mg/kg/day IP for 14 days) did not modify the responsiveness of hippocampal pyramidal neurons to microiontophoretically applied 5-HT, NE and GABA whereas imipramine treatment (5 mg/kg/day IP) increased selectively their sensitivity to 5-HT when compared to indalpine-treated rats. In keeping with its potent reuptake-blocking property, acute IV indalpine produced a marked decrease in the firing rate of dorsal raphe 5-HT neurons (ED_{50} 0.33 mg/kg). The firing rate of dorsal raphe 5-HT neurons was assessed following 2-, 7- and 14-day treatments with indalpine (5 mg/day IP). After 2 days, the firing rate of 5-HT neurons was greatly reduced, after 7 days it had recovered partially and after 14 days it had returned to normal. At this point, the responsiveness of 5-HT neurons to IV LSD, an agonist of the 5-HT autoreceptor, and to microiontophoretically-applied 5-HT was decreased twofold, indicating desensitization of the autoreceptor. In conclusion, it is proposed that long-term treatment with mianserin, as with tricyclic antidepressant drugs and electroconvulsive shocks, increases 5-HT neurotransmission via sensitization of postsynaptic neurons to 5-HT whereas long-term treatment with indalpine, as with zimelidine, results in the same final effect via its presynaptic effect on 5-HT neurons presumably by blocking 5-HT reuptake. These data further support the notion that enhancing 5-HT neurotransmission might have an antidepressant effect.

Key words: Mianserin – Indalpine – Antidepressant drugs – Hippocampus – Dorsal raphe nucleus – Sensitization – Subsensitivity – 5-HT system

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Numerous studies on antidepressant treatments have examined the effect on neurons that are postsynaptic to monoaminergic inputs (Charney et al. 1981). However, given the powerful self-regulatory capacities of monoaminergic neurons (Aghajanian 1978), it is imperative to assess both the postsynaptic modifications and the activity of presynaptic neurons in order to evaluate the net change in neurotransmission produced by a treatment.

Long-term administration of different types of tricyclic antidepressant (TCA) drugs or repeated electroconvulsive treatments (ECT) augments neuronal response to microiontophoretically applied serotonin (5-HT) in various regions of the rat CNS that receive 5-HT input (de Montigny and Aghajanian 1978; Gallager and Bunney 1979; Menkes et al. 1980; Wang and Aghajanian 1980; Menkes and Aghajanian 1981; de Montigny 1984). This enhanced responsiveness of postsynaptic neurons to 5-HT following long-term TCA treatment leads neither to a compensatory decrease in the firing rate of 5-HT neurons nor to modification in the sensitivity of their autoreceptors (Blier and de Montigny 1980). Furthermore, this postsynaptic sensitization can be detected by the endogenous release of 5-HT that results from electrical activation of the ascending 5-HT pathway (Wang and Aghajanian 1980).

Long-term administration of zimelidine, an antidepressant 5-HT reuptake blocker (Ross and Renyi 1977; Åberg and Holmberg 1979; Coppen et al. 1979; Montgomery et al. 1982), prolongs the response of rat hippocampal neurons to the electrical stimulation of their 5-HT afferent pathway without modifying their responsiveness to microiontophoretically-applied 5-HT (de Montigny et al. 1981; Blier and de Montigny 1983). This indicates that long-term zimelidine treatment enhances 5-HT neurotransmission via a presynaptic mechanism, presumably by blocking 5-HT reuptake. During the first few days of zimelidine administration, the firing rate of 5-HT neurons is markedly decreased but returns to normal within 14 days of continuous treatment (Blier and de Montigny 1983). This progressive recovery of firing activity is probably due to desensitization of the 5-HT autoreceptor as suggested by the attenuated response of 5-HT neurons to LSD (Blier and de Montigny 1983).

Thus enhanced 5-HT neurotransmission can result from the following two types of modification: (1) from increased responsiveness of the postsynaptic element with no modification of presynaptic neuron activity, as with TCA drugs; (2) from increased effectiveness of the presynaptic neuron with no modification of postsynaptic responsiveness, as with zimelidine. From this model, the efficacy of 5-HT neuro-

transmission can be estimated by assessing presynaptic neuron activity and postsynaptic neuron responsiveness.

The present experiments were undertaken to assess the effect of long-term treatment with mianserin and indalpine, two antidepressant drugs (Itil et al. 1972; Chazot et al. 1978; Pinder et al. 1980; Guelfi et al. 1981; Montgomery et al. 1983; Shopsin et al. 1983), on the 5-HT system. Mianserin is a tetracyclic drug which interferes neither with 5-HT nor norepinephrine (NE) reuptake processes at clinically relevant dosages (Leonard 1974; Koe 1975). Long-term mianserin administration increases the behavioral syndrome produced by the 5-HT agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), suggesting that mianserin might induce, as do TCA drugs, a postsynaptic sensitization to 5-HT (Friedman et al. 1983). However, this has never been documented by electrophysiological studies. Indalpine is a bicyclic drug which selectively blocks 5-HT reuptake (Le Fur and Uzan 1977). It is devoid of effects on other monoaminergic reuptake processes at clinically relevant doses and has no affinity for pre- or postsynaptic monoaminergic receptors (Le Fur 1983). The effects of mianserin and indalpine on postsynaptic neurons were studied by assessing the response of hippocampal pyramidal neurons to microiontophoretic applications of 5-HT, NE, and γ-aminobutyric acid (GABA) following a 14-day treatment. Their ability to modify the firing rate of dorsal raphe 5-HT neurons was also examined.

Materials and methods

Male Sprague-Dawley rats (150–275 g) were used. For long-term treatments, daily IP injections of saline solution (0.5 ml), mianserin (5 mg/kg) or indalpine (5 mg/kg) were administered.

Recordings from dorsal hippocampus pyramidal neurons

The microiontophoretic experiments were carried out 40–50 h after the last dose of the 14-day treatment. Rats were anesthetized with urethane (1.25 g/kg IP) 1 h prior to the experiment, and mounted in a stereotaxic apparatus. Blank five-barrelled micropipettes were pulled in the conventional manner and the tip broken back to 7–10 µm under microscopic control. The central barrel, filled with a 2 *M* NaCl solution saturated with Fast Green FCF (Fisher, Springfield, NJ, USA), was used for recording. The following solutions were used for microiontophoresis: 5-HT creatinine sulfate (0.002 *M* in 0.2 *M* NaCl, pH 4.0; Sigma, St. Louis, MO, USA); NE bitartrate (0.1 *M*, pH 4.0; Sigma, St. Louis, MO, USA); acetylcholine chloride (ACh, 0.02 *M* in 0.2 *M* NaCl, pH 4.0; Sigma, St. Louis, MO, USA); and GABA (0.02 *M* in 0.02 *M* NaCl, pH 4.0; Calbiochem, Los Angeles, CA, USA). These drugs were retained with a −8 nA current between ejection periods.

Neuronal responsiveness was assessed using the IT_{50} method (de Montigny and Aghajanian 1977). In brief, it consists of determining the charge (current × time) required to obtain a 50% decrease in firing rate of the neuron recorded. Since the number of molecules ejected is proportional to the charge, the more sensitive the neuron the smaller will be the IT_{50}. The ejection current was adjusted to obtain a 50% depression of firing rate within 10–50 s, since the log-concentration of the drug in the tissue increases linearly during that period (Simmonds 1974).

Dorsal hippocampal pyramidal neurons were recorded from the CA_3 region at about 3.5 mm below the surface of the cortex. They were identified by their high-amplitude (0.5–1.2 mV) long duration (0.6–1.0 ms) action potentials and by their characteristic complex spike discharge (Kandel and Spencer 1961). To activate silent or slowly discharging neurons within their physiological range, a small current of ACh was used. Currents of ACh ranging from −8 to +8 nA do not modify the responsiveness of these neurons to 5-HT (Brunel 1983). In order to increase the accuracy of comparisons, the same micropipette was used to determine responsiveness to microiontophoretic applications in two or three rats pretreated with different substances. This procedure eliminates the variance that could be due to the difference in microiontophoretic ejection efficacy from one pipette to the other.

Recording from dorsal raphe 5-HT neurons

Rats were anesthetized with chloral hydrate (400 mg/kg IP), and mounted in a stereotaxic apparatus. Serotonergic dorsal raphe neurons were recorded conventionally with a single-barrelled glass micropipette filled with a 2 *M* NaCl solution saturated with Fast Green FCF or with a five-barrelled micropipette prepared as already described. The micropipette was lowered 0.8 mm anterior to lambda on midline. 5-HT neurons were encountered immediately after passing the ventral border of the cerebral aqueduct (aqueduct of Sylvius). They were identified using the criteria, as defined by Aghajanian (1978), of a low (0.5–2.5 Hz) and regular firing rate and a long-duration (0.8–1.2 ms) positive action potential. Aghajanian and VanderMaelen (1982) contributed an ultimate demonstration of the 5-HT nature of neurons displaying these electrophysiological characteristics in the dorsal raphe nucleus by combining intracellular marking and histofluorescence. A single dose of mianserin or indalpine in a volume of 0.05 ml was administered through a tail vein while recording from a dorsal raphe 5-HT neuron.

To assess the firing activity of 5-HT neurons during the course of indalpine treatment, five electrode tracks in each rat were done in a stereotaxically defined block of tissue (one on midline at 0.8 mm anterior to lambda, two 200 µm lateral on each side and two 200 µm anterior and posterior to the first track). Each descent was terminated 1 mm below the ventral border of the cerebral aqueduct. Each 5-HT neuron encountered was recorded for at least 1 min to determine its firing rate.

LSD is a potent agonist of the 5-HT autoreceptor (Haigler and Aghajanian 1974). LSD (10 µg/kg) was administered IV to controls and rats treated for 14 days with indalpine while recording from a 5-HT neuron to assess the sensitivity of the 5-HT autoreceptor. A Fast Green deposit was left at the last recording site for histological verification from cryostat histological sections.

Results

Mianserin

Hippocampal pyramidal neurons were chosen to study the effects of mianserin on forebrain postsynaptic neurons for the following reasons: (1) they receive both 5-HT and NE projections from the mesencephalic raphe nuclei and the locus coeruleus respectively (Andén et al. 1966; Jones and Moore 1977; Azmitia and Segal 1978; Kohler 1982); (2) the

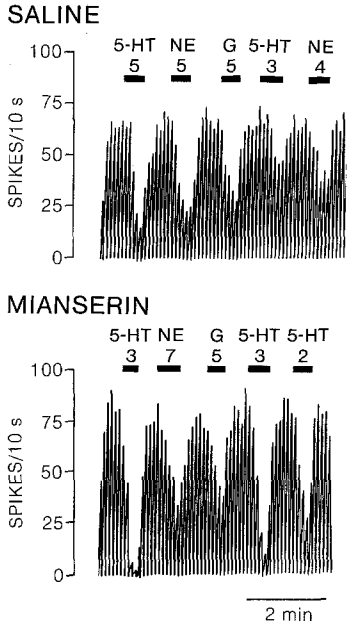

Fig. 1. Integrated firing rate histograms of CA_3 hippocampal pyramidal neurons recorded in a saline- and a mianserin-treated rat (5 mg/kg/day IP for 14 days) showing the response to microiontophoretic applications of 5-HT, NE and GABA. *Solid bars* indicate duration of ejections and currents are given in nanoamperes. Time base applies for both traces

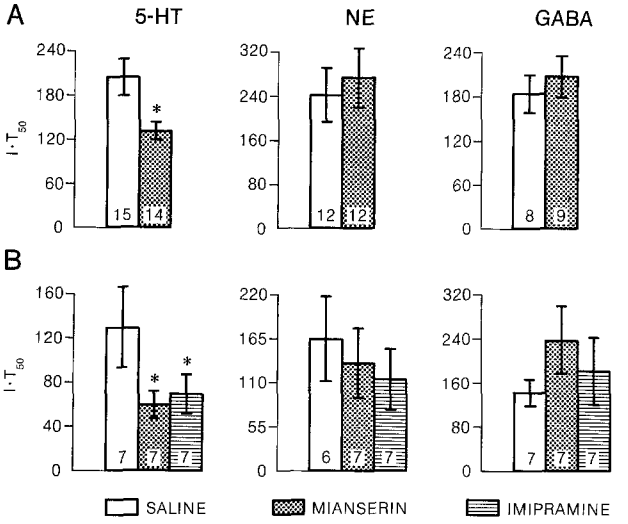

Fig. 2A, B. Mean ± SEM IT_{50} values of CA_3 hippocampal pyramidal neurons for microiontophoretic applications of 5-HT, NE and GABA in rats treated with saline, mianserin or imipramine (5 mg/kg/day IP for 14 days). Experiments were performed 48 h after the last injection using the same micropipette to assess responsiveness for two **A** or three **B** rats. * $P < 0.05$ compared with control values using the two-tailed Student's *t*-test

hippocampus is part of the limbic system, which is generally believed to be involved in emotional behavior; (3) these neurons exhibit an increased responsiveness to 5-HT following long-term TCA or ECT treatments (de Montigny and Aghajanian 1978; Gallager and Bunney 1979; de Montigny et al. 1981; de Montigny 1984).

Figure 1 shows two firing rate histograms of dorsal hippocampus pyramidal neurons obtained with the same micropipette in a control and a mianserin-treated rat. The responsiveness to 5-HT is greater in the mianserin-treated animal whereas the effectiveness of GABA and NE applications are similar in both rats. The results obtained in a first series of experiments comparing saline- and mianserin-treated rats are presented in Fig. 2A. In a second series of experiments, an imipramine-treated group was added as active controls to determine if the degree of sensitization induced by mianserin was comparable to that produced by a TCA. The same micropipette was used to determine responsiveness in each group of three rats. There was no significant difference in responsiveness to 5-HT between the imipramine and the mianserin groups. Responsiveness to 5-HT in both imipramine- and mianserin-treated rats was greater than in controls (Fig. 2B). The efficacy of NE and GABA was unaltered by the treatments (Fig. 2B). The mean current (nA ± SEM) of ACh used to activate silent or slowly discharging neurons did not differ among the three groups (control 0.2 ± 0.5, mianserin -1.0 ± 0.5, imipramine -1.6 ± 0.6).

In order to ascertain that a long-term treatment with mianserin is required to induce sensitization of postsynaptic neurons to 5-HT, as with TCA, the responsiveness of dorsal hippocampus pyramidal neurons to 5-HT, NE or GABA was assessed following 2-day treatments with mianserin (5 mg/kg/day IP) or saline (0.5 ml/day IP). This short-term

Table 1. Responsiveness (IT_{50} ± SEM) of dorsal hippocampus pyramidal neurons to microiontophoretic applications of 5-HT, NE and GABA following 2-day treatment with mianserin (5 mg/kg/day IP). The number of neurons tested is given in parentheses. None of the values of the mianserin group was significantly different from the corresponding value of the control group. The mean currents of ACh used in the control and in the mianserin groups were 2.5 ± 0.4 and 2.2 ± 0.3 nA, respectively

	5-HT	NE	GABA
Control	70 ± 12 (13)	74 ± 9 (13)	307 ± 40 (9)
Mianserin	77 ± 12 (13)	73 ± 10 (13)	349 ± 39 (9)

mianserin treatment failed to alter responsiveness to the three neurotransmitters tested (Table 1).

The ability of mianserin to modify the firing rate of 5-HT neurons was studied in seven rats, i.e. an IV dose of 5 mg/kg was administered while recording from a 5-HT neuron in the dorsal raphe nucleus. Three animal died shortly after the injection. Figure 3 illustrates the response of a 5-HT unit to the acute IV administration of two successive doses of 5 mg/kg mianserin. In the three other experiments, 5 mg/kg mianserin also failed to reduce the firing rate of 5-HT neurons in the dorsal raphe nucleus. In three of these four experiments, this dose of mianserin produced a slight transient increase in firing activity. LSD (10 µg/kg IV) was injected following 10 mg/kg mianserin in two experiments. In both instances, LSD failed to produce the complete cessation of firing usually obtained in control rats (see Fig. 9A).

Indalpine

In a first series of experiments, the responsiveness of hippocampal pyramidal neurons to 5-HT, NE and GABA

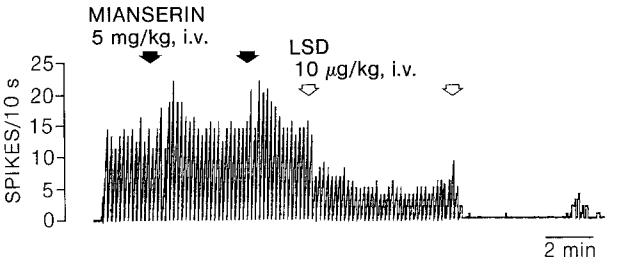

Fig. 3. Integrated firing rate histogram of a dorsal raphe 5-HT neuron showing the response to IV injections (*arrows*) of mianserin and LSD

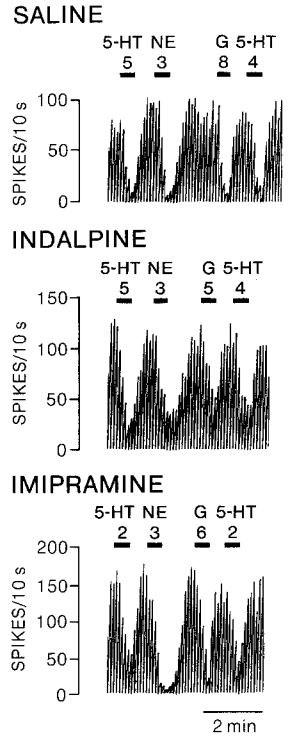

Fig. 4. Integrated firing rate histograms of CA_3 hippocampal pyramidal neurons showing their response to microiontophoretic applications of 5-HT, NE and GABA in rats treated with saline, indalpine or imipramine (5 mg/kg/day IP for 14 days). All experiments were carried out 48 h after the last dose. The last two traces were obtained using the same micropipette

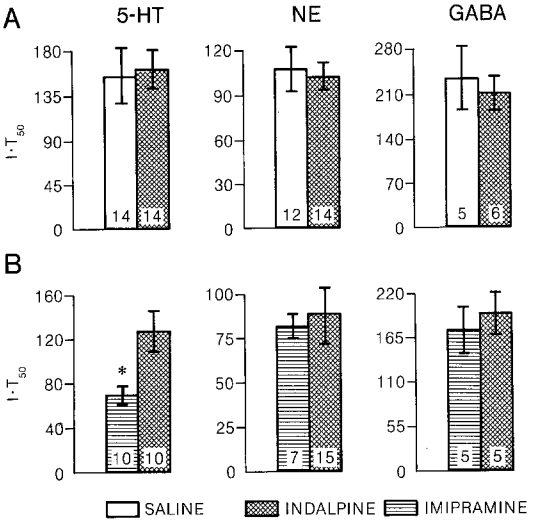

Fig. 5. Mean ± SEM IT_{50} of CA_3 hippocampal pyramidal neurons to microiontophoretic applications of 5-HT, NE and GABA in pairs of indalpine- and saline-treated rats **A**, and in pairs of indalpine- and imipramine-treated rats **B**. Both drugs were administered at a dose of 5 mg/kg/day IP for 14 days. * $P < 0.01$ compared with the imipramine group using the two-tailed Student's *t*-test

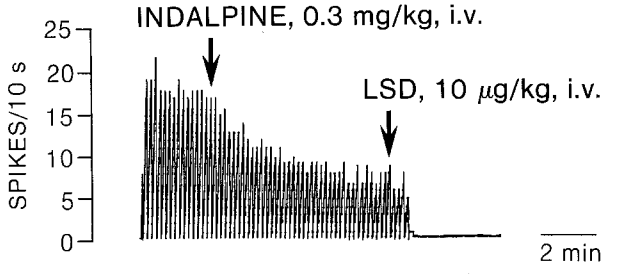

Fig. 6. Depression of firing rate of a dorsal raphe 5-HT neuron by IV injections (*arrows*) of indalpine and LSD in an untreated rat

was assessed in pairs of saline- and indalpine-treated animals using the same micropipette. The responsiveness to microiontophoretic applications of these three substances was not modified by the indalpine treatment (Figs. 4 and 5A). In order to further ascertain the inability of indalpine to induce sensitization to 5-HT, a second series of experiments was carried out in pairs of animals treated with indalpine or imipramine. The IT_{50} values for 5-HT were smaller in the imipramine than in the indalpine group but those for NE and GABA were not different (Fig. 5B). The mean current (nA ± SEM) of ACh used in the indalpine group was not different from that used in the control rats (1.0 ± 0.3 versus 1.4 ± 0.3 respectively), but less ACh was necessary to activate these neurons to their physiological firing rate in this imipramine group (0.4 ± 0.3).

Various TCA depress the firing rate of 5-HT neurons (Sheard et al. 1972; Scuvée-Moreau and Dresse 1979). This is attributable to the TCA property of 5-HT reuptake blockade, since their potency in exerting this electrophysiological effect closely parallels their potency in blocking 5-HT reuptake (Scuvée-Moreau 1981). This decrease in firing is attributable to reuptake blockade in the raphe itself, since Mosko et al. (1977) showed that a transection immediately rostral to this nucleus does not prevent the inhibitory effect of chlorimipramine on the firing rate of 5-HT neurons. The firing rate histogram of Fig. 6 exemplifies the response of a 5-HT neuron to the IV administration of indalpine (0.3 mg/kg produced a 58% inhibition within 4 min). The complete cessation of firing produced by the subsequent injection of 10 μg/kg LSD confirms the 5-HT nature of the recorded neuron. The dose-response curve of Fig. 7 summarizes the results obtained in nine animals. The high degree of dose-response relationship reflects the precision of the model. From these data an ED_{50} of 0.33 ± 0.02 mg/kg was calculated.

The effect of indalpine on reuptake manifests itself within minutes whereas the antidepressant effect is not detectable for several days (Chazot et al. 1978; Guelfi et al. 1981; Shopsin et al. 1983). Facing this apparent discrepancy, we undertook another series of experiments to determine the

activity of 5-HT neurons in the dorsal raphe nucleus following repeated administration of indalpine.

At 24 h after the last dose of a 2-day treatment with indalpine, the number of spontaneously active 5-HT neurons was greatly reduced and firing rate was much lower. After 7 days of treatment, the number of spontaneously active 5-HT neurons per tract was again normal but their firing rate, though higher than after 2 days, was still significantly lower than in controls. After 14 days of treatment, whether recordings were continued 24 or 48 h after the last dose, both the number of spontaneously active 5-HT neurons and their mean firing rate had normalized (Table 2, Fig. 8).

Since the 5-HT autoreceptor plays an important role in regulating the rate of discharge of 5-HT neurons (Aghajanian 1978), its sensitivity was assessed, after a 14-day treatment with indalpine, by using LSD, a potent agonist of the 5-HT autoreceptor (Haigler and Aghajanian 1974). Figure 9 shows the complete and sustained suppression of firing of a dorsal raphe 5-HT neuron by 10 μg/kg LSD IV in an untreated rat, and the much attenuated sensitivity to LSD of a 5-HT neuron recorded in an animal pretreated with indalpine for 14 days. In all control animals, the administration of 10 μg/kg LSD produced a 100% reduction of the firing activity of 5-HT neurons ($N = 6$) whereas, in animals treated with indalpine for 14 days, the same dose produced only a 53% ± 6% ($N = 6$) reduction of firing rate ($P < 0.001$, two-tailed Student's t-test). The ED_{50} of LSD in indalpine-treated rats would thus be increased at least 2.5-fold, since its ED_{50} in naive rats is approximately 4 μg/kg (Blier and de Montigny 1980).

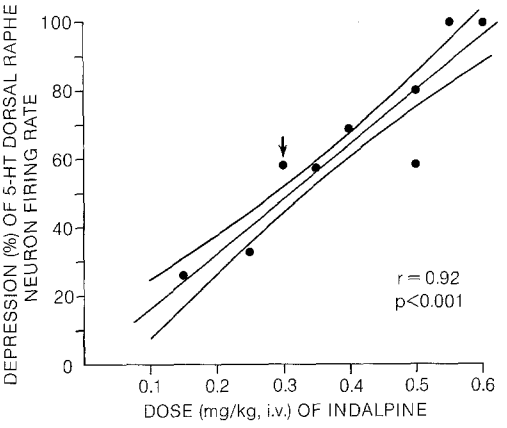

Fig. 7. Relationship between the degree (%) of depression of firing rate of 5-HT neurons and the injected dose of indalpine. Each point represents the response of one neuron to one dose given in a 0.05 ml volume to one rat. The curved lines represent the 95% confidence intervals for the prediction of y given the value of x. The *arrow* identifies the experiment presented in Fig. 6

Table 2. Activity of 5-HT neurons during repeated indalpine treatment (5 mg/kg/day IP). The time interval between the last injection and electrophysiological experiments is given in parentheses

	Duration of treatment	Number ±SEM of spontaneously active 5-HT neurons recorded per 1 mm electrode tract in the dorsal raphe nucleus	Number of tracts
Control		4.3 ± 0.5	17
Indalpine	2 days (24 h)	2.5 ± 0.7*	20
	7 days (24 h)	4.3 ± 1.0	14
	14 days (24 h)	4.5 ± 0.6	15
	14 days (48 h)	5.1 ± 0.7	7

* $P < 0.05$ compared with control values using two-tailed Student's t-test

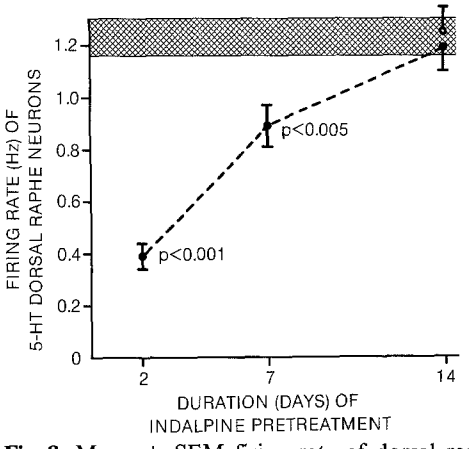

Fig. 8. Mean ± SEM firing rate of dorsal raphe 5-HT neurons recorded 24 h after a 2-, 7- or 14-day treatment with indalpine (5 mg/kg/day IP). The *hatched* area represents the mean firing rate ± SEM. The *open circle* represents the firing rate of 5-HT neurons 48 h after the last dose

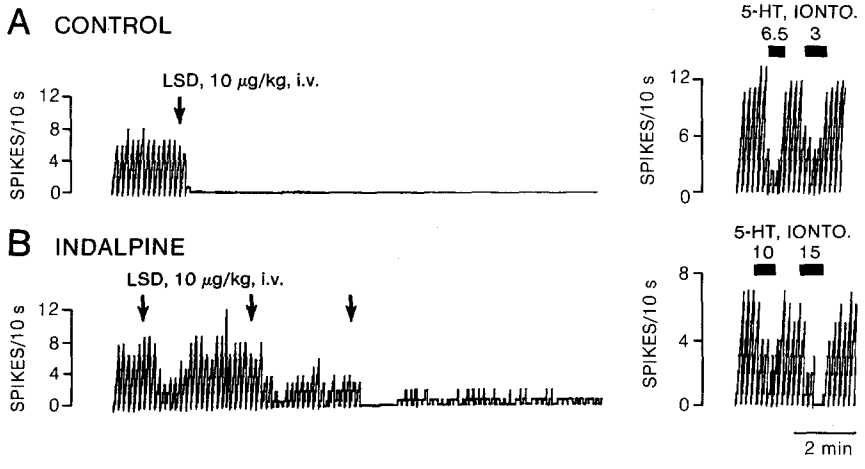

Fig. 9
Integrated firing rate histograms of dorsal raphe 5-HT neurons recorded in naive and indalpine-treated rats (5 mg/kg/day IP for 14 days) showing their response to IV administrations of LSD and to microiontophoretic applications of 5-HT

In order to rule out the remote possibility that the pharmacokinetics of LSD might have been modified by the indalpine treatment, 5-HT autoreceptor sensitivity was assessed directly by applying 5-HT microiontophoretically on dorsal raphe 5-HT neurons following a 14-day indalpine treatment. The mean IT_{50} value for 5-HT in the indalpine-treated rats was twice that obtained in control rats (244 ± 52, $N = 10$ versus 122 ± 30, $N = 12$; $P < 0.05$, two-tailed Student's t-test).

Discussion

The present results show that long-term, but not short-term, mianserin administration induces a sensitization of forebrain neurons to 5-HT. This is consistent with the previously reported enhancement of the behavioral responsiveness to 5-MeODMT, a 5-HT agonist, by long-term but not acute administration of this drug (Friedman et al. 1983). It is remarkable that, although the response of forebrain neurons to microiontophoretically applied 5-HT and the behavioral effect of systemic administration of 5-HT agonists are mediated by different postsynaptic 5-HT receptors (Aghajanian 1981; VanderMaelen and Aghajanian 1982), there is a strong correlation between data obtained by both approaches. Indeed, thus far the three types of antidepressant treatments shown to induce sensitization of forebrain neurons to 5-HT applied microiontophoretically (i.e. TCA, ECT and mianserin) also enhance the behavioral effect of 5-HT agonists administered systemically (Evans et al. 1976; Friedman et al. 1983). Since Menkes et al. (1980) demonstrated sensitization of facial motoneurons to 5-HT by long-term TCA treatments, one may assume that ECT and mianserin also enhance the responsiveness of motoneurons to 5-HT in parallel to their effect on forebrain neurons.

Mianserin has been shown, in radioligand-binding studies, to bind to 5-HT_2 sites in cerebral cortex and hippocampus (Peroutka and Snyder 1980; Blackshear and Sanders-Bush 1982). However, the possibility that increased neuronal responsiveness to 5-HT following long-term treatment with mianserin could result from chronic blockade of 5-HT_2 sites in the hippocampus is unlikely, since sensitization to 5-HT is also obtained with iprindole, a TCA which has a very low affinity for this site (de Montigny and Aghajanian 1978; Peroutka and Snyder 1980). Furthermore, a decrease in the number of 5-HT_2 sites has been reported following long-term treatments with either TCA (Kellar et al. 1981) or mianserin (Blackshear and Sanders-Bush 1982). As yet, the physiological significance of the 5-HT_2 binding site is unclear. That this site might not be implicated in mediating the therapeutic effect of antidepressant treatment is suggested by two observations: (1) TCA and ECT, which both reverse depressive symptoms, exert opposite effects on the number of 5-HT_2 binding sites (Kellar et al. 1981); (2) acute mianserin administration reduces the number of 5-HT_2 sites (Blackshear and Sanders-Bush 1982) whereas long-term treatment is required to produce an antidepressant effect.

The molecular mechanism responsible for neuronal sensitization to 5-HT by long-term mianserin or TCA treatments remains unknown. A simple modification of the membrane receptors seems unlikely. It is possible that the gain of neuronal transducing mechanisms might be set at a higher level by long-term administration of these drugs.

In the present study, acute administration of mianserin (up to 10 mg/kg IV) was ineffective in decreasing the firing rate of 5-HT neurons (Fig. 1). Scuvée-Moreau (1981) reported an ED_{50} of 9 mg/kg for mianserin using the same model used here, with the exception that the drug was injected over several minutes. Given the toxicity of acute IV administration of mianserin that is shown in the present study, one could wonder if the slowing of 5-HT neuron firing observed by Scuvée-Moreau (1981) with slow IV infusion of very high doses of mianserin could not be attributable to a toxic effect rather than to a specific serotonergic effect of the drug. At any rate, the relative ineffectiveness of mianserin in depressing 5-HT neuron firing rate is fully consistent with its very weak activity as a 5-HT reuptake blocker (Leonard 1974; Koe 1975). The transient increase in firing rate of 5-HT neurons and the reduced efficacy of LSD following mianserin administration would both be consistent with the reported antagonistic activity of mianserin at the 5-HT autoreceptor (Ennis and Cox 1982; Göthert and Schlicker 1983). This phenomenon might not be clinically relevant, since the brain concentration of mianserin produced by an acute IV injection of 5 mg/kg is probably much higher than that attained in patients treated with this drug.

Mianserin potentiates NE release evoked by K^+ (Nickolson and Weiringa 1981) or electrical stimulation (Baumann and Maitre 1977) in brain slices, suggesting that it blocks α_2 presynaptic receptors. Consistent with these biochemical data, the depressant effect of microiontophoretically applied NE on the firing rate of locus coeruleus neurons is prevented by the concurrent ejection of mianserin (Svensson et al. 1981). The clinical relevance of the α_2-blocking property of mianserin is questionable, since most TCA are weak α_2-receptor antagonists (Maggi et al. 1980). At any rate, neither the firing rate of NE neurons in the locus coeruleus nor the sensitivity of their α_2-somatic autoreceptors are modified by long-term treatment with mianserin (Scuvée-Moreau and Svensson 1982).

The responsiveness of hippocampal pyramidal neurons to NE was not modified by any of the antidepressant drugs used in the present study (Figs. 2 and 5). This contrasts the reduction of cyclic AMP response to NE in limbic forebrain following long-term treatment with either mianserin or zimelidine (Mishra et al. 1980). Such an apparent discrepancy has already been disclosed with other types of antidepressant treatments, such as TCA and ECT (de Montigny and Aghajanian 1978; Sulser 1979; de Montigny 1984). It is thus likely that a modification of the NE-sensitive adenylate cyclase system does not alter the effect of microiontophoretically-applied NE on the spontaneous and ACh-induced firing activity of hippocampal pyramidal neurons. However, this does not dismiss the possibility that other electrophysiological effects of NE, such as the blockade of spike frequency accomodation (Madison and Nicoll 1982), could be modified by long-term antidepressant treatments.

Chronic administration of indalpine, in contrast to imipramine, does not modify the responsiveness of hippocampal pyramidal neurons to 5-HT (Fig. 5). Together with similar results obtained with zimelidine (de Montigny et al. 1981; Blier and de Montigny 1983), this indicates that long-term 5-HT reuptake blockade does not result in a of the postsynaptic moiety in the hippocampus.

The remarkable potency of indalpine in depressing the firing rate of 5-HT neurons is consistent with its potent 5-HT reuptake blocking property (Le Fur et al. 1978). The

significant decrease in firing rate of 5-HT neurons after 2 days of indalpine treatment was therefore not unexpected. After a 14-day treatment, the activity of 5-HT neurons returned to normal levels, at which point the responsiveness to IV LSD and to microiontophoretically applied 5-HT were both markedly decreased (Fig. 9), indicating a desensitization of the 5-HT autoreceptor, presumably resulting from its exposure to a greater concentration of 5-HT during sustained reuptake blockade. Hence the progressive restoration of the firing rate of 5-HT neurons during the course of indalpine treatment, in spite of a sustained 5-HT reuptake blockade, is likely to be due to the progressive freeing of the 5-HT neurons from their powerful autoregulatory control as their autoreceptor desensitizes. The time-course of these events can account for the delayed therapeutic effect of indalpine in major depression: Sustained 5-HT reuptake blockade cannot yield a greatly increased neurotransmission until 5-HT neurons resume their normal firing activity.

In conclusion, these experiments bring further support to the notion that enhanced 5-HT neurotransmission might have an antidepressant effect whether it is achieved presynaptically via 5-HT reuptake blockade, as with indalpine and zimelidine, or postsynaptically via a sensitization of the target neuron as with TCA, ECT and mianserin.

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