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Eltoprazine Suppresses Hyperpolarizing Responses to Serotonin in Rat Hippocampus

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ABSTRACT

In this study we report the effects of eltoprazine, a phenylpiperazine derivative with high affinity for 5-hydroxytryptamine$_1$ ($5HT_1$) binding sites, on membrane properties of hippocampal neurons. Intracellular recordings were made from cornus ammoni-1 pyramidal neurons in rat hippocampal slices. Responses to eltoprazine were compared with 5HT-induced responses. Superfusion with 5HT induced a dose-dependent hyperpolarization of the membrane accompanied by a resistance decrease. Eltoprazine evoked membrane changes that were similar to but much weaker than those induced by 5HT. Both the 5HT- and eltoprazine-evoked membrane hyperpolarizations were largely suppressed in the presence of spiperone. The eltoprazine-induced effects persisted in the presence of tetrodotoxin and tetraethylammonium and also when haloperidol and phentolamine were added to the medium, indicating that the small agonistic effects of eltoprazine are not due to an indirect activation of dopamine or *alpha* adrenergic receptors. Superfusion with eltoprazine furthermore resulted in a marked reduction of the response to concomitantly applied 5HT. Dose-response curves for 5HT were shifted to the right in the presence of eltoprazine, while the maximal response was diminished. Hyperpolarizations induced by baclofen, which presumably activates the same K^+ conductance as 5HT, were not significantly reduced by eltoprazine. Our data, added to the previously demonstrated high affinity of eltoprazine for $5HT_1$ sites, suggest that in the hippocampal cornus ammoni-1 area eltoprazine acts as a partial $5HT_1$ agonist with a relatively low intrinsic activity but a considerable potency to suppress hyperpolarizing responses to 5HT.

Eltoprazine (DU 28853) is a recently developed bicyclic heteroaryl piperazine with specific anti-aggressive activity (Olivier *et al.*, 1987). It was found that this compound decreases the offensive components of agression in mice, rats, pigs and monkeys while leaving nonaggressive social interactions intact. The anti-aggressive activity was not accompanied by sedation or muscle relaxation.

There are several indications that the behavioral effects of eltoprazine are at least partly mediated by 5HT receptors in the brain. Thus, binding studies revealed that eltoprazine binds with a high affinity to $5HT_{1A}$, $5HT_{1B}$ and $5HT_{1C}$ receptors. Only moderate affinity was found for *beta* and *alpha*-1 adrenergic receptors, while the affinity for $5HT_2$ and other receptors was extremely low (Olivier *et al.*, 1987; Schipper *et al.*, 1987). Eltoprazine reduced the *in vitro* release of 5HT, acting as a partial agonists on the presynaptically localized $5HT_{1B}$ site with ~40% intrinsic activity (Olivier *et al.*, 1987; Schipper *et al.*, 1987). The effect of eltoprazine on the $5HT_{1B}$ site is likely to contribute to its anti-aggressive activity (Olivier *et al.*, 1987), but interactions with the $5HT_{1A}$ sites might also contribute to the overall behavioral profile of the compound. In the present study we have used intracellular electrophysiological recording techniques to investigate the mechanism of action of eltoprazine on a population of $5HT_{1A}$ sites in the rat brain.

We selected the CA1 area of the rat hippocampus to test the effect of eltoprazine since this region displays a high density of $5HT_{1A}$ binding sites (Deshmukh *et al.*, 1983; Köhler, 1984; Marcinkiewicz *et al.*, 1984; Pazos and Palacios, 1985). Previous electrophysiological investigations have shown that 5HT induces at least three different actions on CA1 pyramidal neurons in hippocampal slices (Jahnsen, 1980; Segal, 1980; Andrade and Nicoll, 1987a; Colino and Halliwell, 1987). The most frequently observed action is a rapid, nondesensitizing membrane hyperpolarization accompanied by a decrease in membrane resistance. Current and voltage clamp studies indicated that an increase in K^+ conductances underlies the hyperpolarization. This action of 5HT can be mimicked by compounds with high affinity for the $5HT_{1A}$ sites, e.g., 8OHDPAT, buspirone and ipsapirone (Andrade and Nicoll, 1987a; Wu *et al.*, 1988); yet, these compounds also suppress the 5HT responses to some degree (Colino and Halliwell, 1986, 1987; Andrade and Nicoll, 1987b). As there are currently no selective $5HT_{1A}$ antagonists

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ABBREVIATIONS: 5HT, 5-hydroxytryptamine, serotonin; CA, cornus ammoni; ACSF, artificial cerebrospinal fluid; TTX, tetrodotoxin; TEA, tetraethylammonium; 8OHDPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin.

available, the hyperpolarizing response to 5HT has been tentatively assigned to an action on the $5HT_{1A}$ receptor. In addition to the hyperpolarizing response, 5HT also evoked a slow depolarizing response which so far could not be linked to any of the known 5HT receptor subtypes (Andrade and Nicoll, 1987a; Colino and Halliwell, 1987). Colino and Halliwell (1987) found that particularly in the dorsal hippocampus the hyperpolarizing response to 5HT predominates. We therefore examined the ability of eltoprazine to mimic or block the hyperpolarizing response induced by 5HT in CA1 neurons of the dorsal hippocampus *in vitro*.

Methods

Hippocampal slices were prepared from male, Wistar rats (100 to 200 g). In a few experiments we used adrenalectomized instead of intact rats. The data obtained in these experiments were only incorporated after we established that the direction of the 5HT or eltoprazine responses was not affected by this operation. After decapitation, the brain was quickly removed from the skull and dipped in ice-cold (4°C), oxygenated (95% O_2 + 5% CO_2) ACSF of the following composition (millimolar): NaCl, 120; KCl, 3.5; NaH_2PO_4, 1.25; $MgSO_4$, 1.3; $CaCl_2$ 2.5; $NaHCO_3$, 25; and glucose, 10. One hippocampal lobe was dissected and 350 μM thick, transverse hippocampal slices were cut on a McIlwain tissue chopper. Subsequently, the slices were incubated in a recording chamber (Brown and Halliwell, 1981) and submerged in ACSF of 32–33°C. Eltoprazine [1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine HCl, Duphar B.V., Weesp], 5HT-creatinine sulfate (Sigma Chemical Company, St. Louis, MO) or baclofen (Ciba-Geigy, Summit, NJ) were applied in known concentrations to the slice by changing the standard ACSF to one which differed only in its content of the drug. When dose-response curves were constructed, we started by testing the lowest concentration and successively tested higher concentrations. We waited for at least 10 min between the testing of successive doses, so that membrane properties were restored to the pretreatment level before each test. In those experiments where eltoprazine was tested for its antagonistic properties, and in the experiments with TTX, TEA (Sigma), spiperone (Duphar, 5 μM dissolved in 0.5% ethanol) or haloperidol (Sigma)/phentolamine (Ciba/Geigy), the drugs were perfused for at least 15 min before the agonist was tested. All drugs were made up immediately before testing.

Intracellular recordings of CA1 neurons were made with 3 M KCl- or 4 M KAc-filled microelectrodes (impedances 50–90 and 80–150 megohms, respectively). The signals were amplified with an Axoclamp-2A amplifier and displayed on a 1425 Gould digital storage oscilloscope. The membrane potential and applied current were registered on a Gould 2200 chart recorder and in some cases on a Vetter videocassette instrumentation recorder for later analysis. Only neurons displaying resting membrane potentials of at least −55 mV, spike amplitudes of at least 80 mV and stable membrane characteristics for more than 30 min were included in this study. Membrane resistance was monitored continuously by passing current pulses (0.1–0.3 nA, 150 msec, 0.2 Hz) through the recording pipette while the bridge balance was adjusted.

The drug-induced membrane effects were calculated by comparing membrane potential and resistance during drug treatment with pretreatment properties. Changes in membrane properties induced by 5HT alone or 5HT in the presence of eltoprazine were statistically evaluated with the Student's *t* test.

Results

In total, we recorded from 60 neurons in the CA1 pyramidal cell layer of the hippocampus. The neurons included in this study displayed typical characteristics of CA1 pyramidal neurons as described by others (Schwartzkroin, 1975, 1976), *e.g.*, accommodation of cell firing during a 500 ms cathodal current pulse and a low spontaneous firing rate. The resting membrane potential of the neurons was −65.8 ± 0.5 mV (mean ± S.E.M.) and their input resistance amounted to 45.2 ± 1.8 megohms.

Bath-applied 5HT hyperpolarized all 49 CA1 neurons tested, while the membrane resistance was decreased (see example in fig. 1A). Dose-response curves were constructed by recording membrane properties of the neurons with increasing concentrations of 5HT. As shown in figure 2, the lowest effective 5HT concentration on both membrane potential and resistance was 1 μM. Maximal changes in membrane potential (−8.8 ± 0.4 mV, n = 23) and membrane resistance (42.0 ± 1.9%, n = 23) were obtained with 30 μM 5HT. Similar responses to 5HT have been previously assigned to activation of $5HT_{1A}$ receptors in the CA1 region of the hippocampus (Andrade and Nicoll, 1987a; Colino and Halliwell, 1987).

We subsequently compared the action of eltoprazine on CA1 neurons with the 5HT-induced responses obtained in the same neurons (see example in fig. 1A). It appeared that compared to 5HT, eltoprazine induced only small changes in the membrane potential (<3.0 mV) and resistance (~10%), even when it was applied in a high concentration (fig. 2). With 30 μM eltoprazine we recorded a membrane hyperpolarization of −2.8 ± 0.5 mV and reduction in input resistance of 11.6 ± 2.1% (mean ± S.E.M., n = 14). Current-voltage curves indicate that the reversal potential for both the 5HT-induced hyperpolarization and the much weaker membrane effect of eltoprazine is ~−90mV, close to E_k in these neurons (see example in fig. 3). In the presence of 1 μM TTX and 5 mM TEA changes in membrane potential (−2.4 ± 0.9 mV, n = 5) and input resistance (16.2 ± 4.0%, n = 5) induced by 30 μM eltoprazine were not significantly different from the responses obtained in control ACSF, supporting a postsynaptic mechanism of action for eltoprazine (see example in fig. 1B). We also investigated whether the hyperpolarization by eltoprazine could be due to an indirect activation of dopaminergic or *alpha* adrenergic receptors, since the latter receptors have been reported to mediate hyperpolarizing responses in CA1 neurons (Bernardo and Prince, 1982; Gribkoff and Ashe, 1984; Madison and Nicoll, 1986a). The changes in membrane potential induced by 30 μM eltoprazine in an ACSF buffer containing 1 μM haloperidol and 10 μM phentolamine appeared to be slightly reduced (−1.5 ± 0.4 mV, n = 6), but the effects on membrane resistance did not differ from the responses in the control ACSF buffer (14.5 ± 3.3%; not shown). Finally, we tested if the hyperpolarizing responses to 5HT (10 μM) and eltoprazine (30 μM) were suppressed in the presence of the nonselective $5HT_{1A}$ antagonist spiperone, as was reported previously for 5HT (Andrade *et al.*, 1986). We found that both the 5HT- and eltoprazine-induced changes in membrane potential (−5.0 ± 0.9 and −3.2 ± 1.1 mV, n = 5 respectively) and input resistance (23.4 ± 1.9 and 13.2 ± 3.5%) were markedly reduced after at least a 20-min perfusion of 5 μM spiperone (membrane potential: 0.4 ± 1.1 and −0.9 ± 0.5 mV; resistance: 6.0 ± 3.4 and 3.8 ± 2.6% for 5HT and eltoprazine, respectively).

We next investigated if eltoprazine could antagonize the hyperpolarizing responses to 5HT in the CA1 area. It was found that when eltoprazine was tested concomitantly with 5HT, it displayed a considerable potency to block the 5HT-evoked hyperpolarizations (see example in fig. 4). In most neurons the 5HT response remained depressed for more than 30 min after washout of eltoprazine was started. Dose-response curves for 5HT were made under control conditions, in the presence of eltoprazine and, if possible, after washout of eltoprazine (fig.

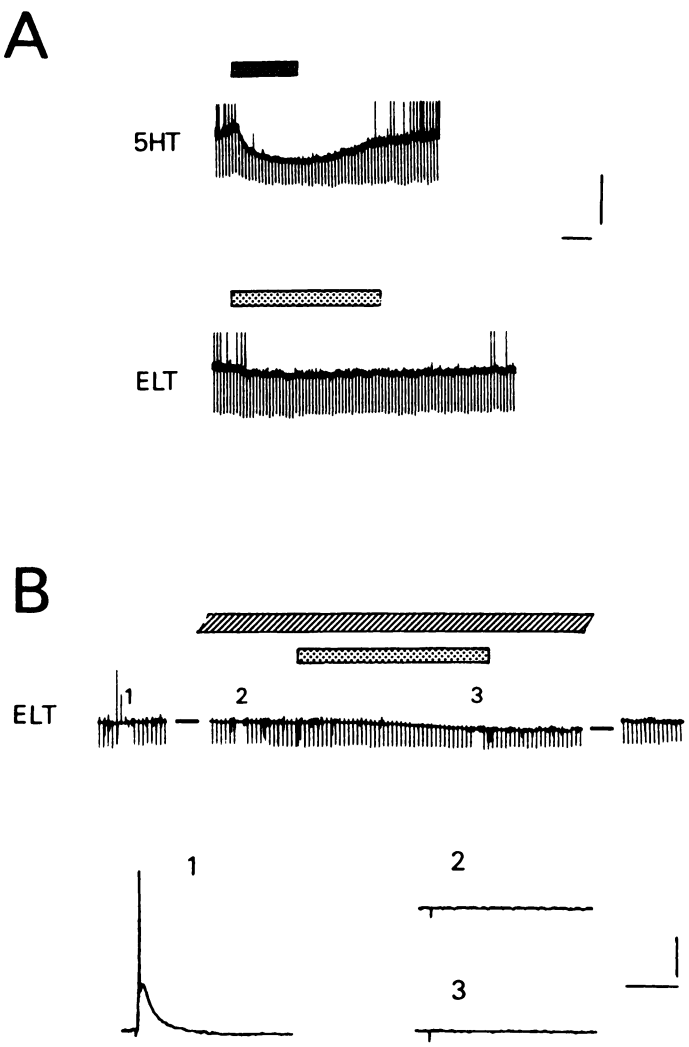

Fig. 1. A. Responses of a CA1 pyramidal neuron to 10 μM 5HT and 10 μM eltoprazine (ELT). During application of 5HT (dark stippled bar, upper panel) the membrane potential is temporarily hyperpolarized and spontaneous firing reduced. The membrane resistance, estimated from the negative voltage deflections evoked by constant current pulses (0.2 nA, 150 msec, 0.2 Hz) is decreased by 5HT. Superfusion with eltoprazine (grey stippled bar, lower panel) only slightly hyperpolarizes the cell, but still reduces the sponaneous firing of the cell. B. Response of a CA1 pyramidal neuron to 30 μM eltoprazine, while 1 μM TTX and 5 mM TEA (hatched bar) are added to the superfusion medium. At three moments, indicated in the record by the numbers 1, 2, and 3 we recorded the response to monosynaptic activation of the neuron induced by a 100 μA current pulse (150 μsec duration), delivered through a bipolar stimulation electrode placed in the stratum radiatum-moleculare. While the synaptic response was completely suppressed during superfusion with TTX and TEA (record 2 and record 3) when compared to the response before treatment (record 1), eltoprazine still induced a small hyperpolarization of the membrane accompanied by a decrease in resistance, similar to the response shown in A. First and second gaps in the record represent intervals of 7 and 9 min, respectively. Calibration bars in upper right corner represent 10 mV (vertical) and 1 min (horizontal) for the voltage trace in A, and 20 mV and 1 min for the upper voltage trace in B. Calibration bars in the lower right corner represent 20 mV (vertical) and 100 ms (horizontal) for the three synaptic responses in B.

5). With a low concentration (1 μM) of eltoprazine the average responses to 10 or 30 μM 5HT were slightly reduced, although these effects were not statistically significant. At higher doses, however, eltoprazine shifted the dose-response curve for 5 HT to the right, while it also reduced the maximal response. It appeared that eltoprazine in particular affected the $5HT_{1A}$

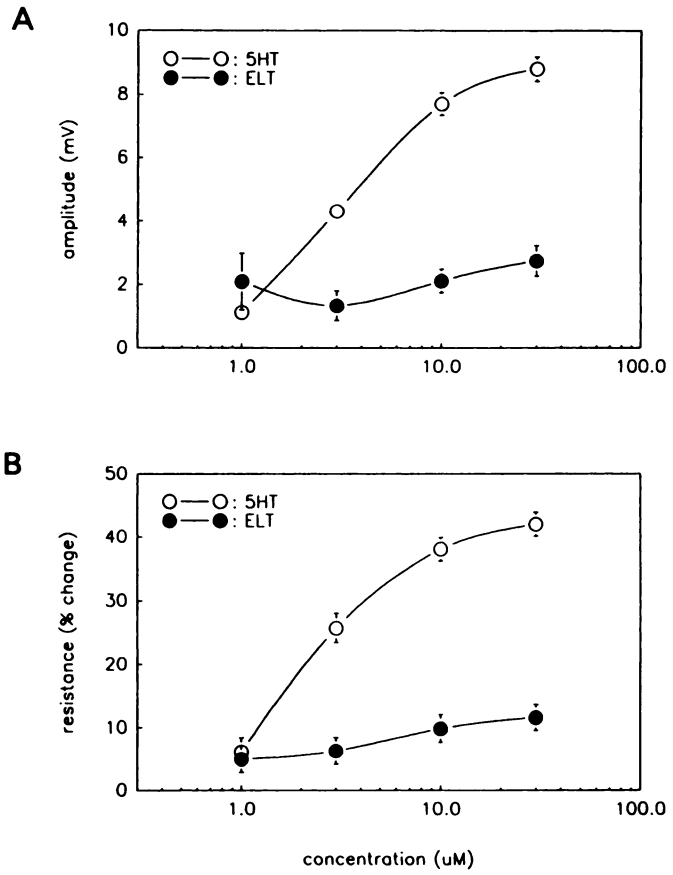

Fig. 2. Dose-response curves for the membrane hyperpolarization (A) and change in resistance (B) induced by 5HT or eltoprazine (ELT). Whenever possible we established the responses to 1 (n = 17), 3 (n = 26), 10 (n = 39) and 30 (n = 23) μM 5HT in each neuron (n = 49). Subsequently we tested the effect of 1 (n = 5), 3 (n = 9), 10 (n = 18) or 30 (n = 14) μM eltoprazine in the same cell. The symbols represent the mean ± S.E. of the mean of all observations.

receptor-mediated hyperpolarizing responses in CA1 neurons. Thus, in addition to the hyperpolarizing responses, 5HT also induced a depolarizing phase in part of the CA1 neurons (1.0 ± 0.3 mV, n = 15, for 10 μM 5HT) which was associated with a small increase in membrane resistance (6.1 ± 1.5% increase, for 10 μM 5HT) and a less efficient spike accommodation in response to a 0.5 nA depolarizing pulse of 500 ms (from 4.8 ± 0.6 spikes before 5HT to 9.8 ± 1.6 spikes immediately after 5HT, n = 4). The depolarizing phase of the 5HT-response was not reduced in the presence of 10 to 30 μM eltoprazine (1.3 ± 0.2 mV and 6.9 ± 1.1%, n = 15, for 5HT-evoked changes in membrane potential and resistance, respectively; spike accommodation from 4.0 ± 0.5 to 7.8 ± 1.8 spikes), although the hyperpolarizing responses to 5HT in the same group of neurons were largely (~50%) suppressed by eltoprazine. To avoid the appearance of a large depolarizing phase in the 5HT response which becomes more prominent with increasing 5HT concentrations, we did not test 5HT concentrations exceeding 30 μM.

As eltoprazine itself decreased the membrane resistance to some extent, we tested the possibility that eltoprazine might reduce 5HT responses through shunting of the K^+ channels rather than through an action on the receptor. Recently, Nicoll and co-workers (Andrade *et al.*, 1986) have shown that the $GABA_b$ agonist baclofen activates the same subset of K^+ channels in hippocampal CA1 neurons as does 5HT by hyperpolar-

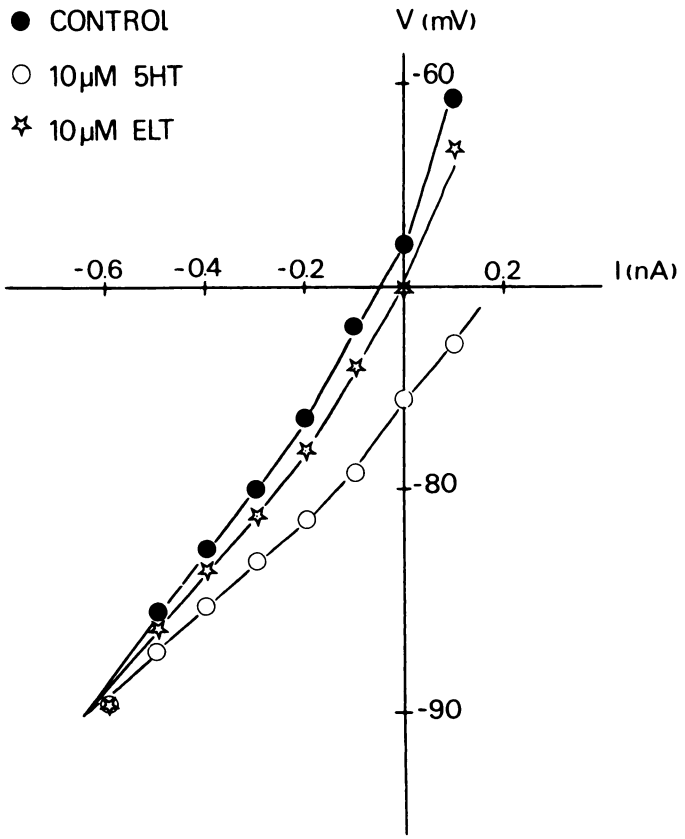

Fig. 3. Current-voltage curve under control condition, during superfusion with 10 μM 5HT or with 10 μM eltoprazine (ELT). The curves were constructed by calculating the voltage deflections induced by current pulses (150 msec) of +0.1 to −0.6 nA. During serotonin and eltoprazine administration, the membrane potential was brought to the pretreatment level by injection of a steady positive direct current before the current-voltage curves were established, as described previously (Joëls *et al.*, 1987).

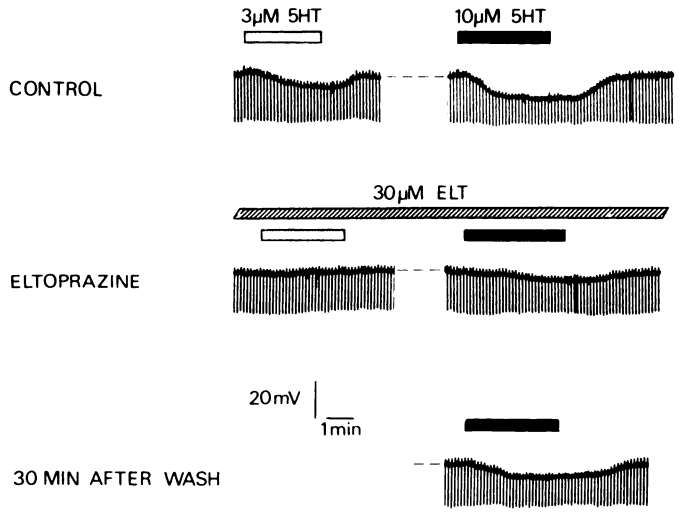

Fig. 4. The effect of eltoprazine (ELT, 30 μM) on responses to concomitantly applied 5HT (3 and 10 μM). Under control conditions, both 3 and 10 μM 5HT induced a hyperpolarization of the membrane with a decrease in resistance. In the presence of eltoprazine, the response to 3 μM 5HT was blocked while the effect of 10 μM was markedly reduced. Thirty minutes after washout of eltoprazine, there was only partial recovery of the response to 10 μM 5HT. Repeated application of 5HT under control conditions did not result in desensitization of the response.

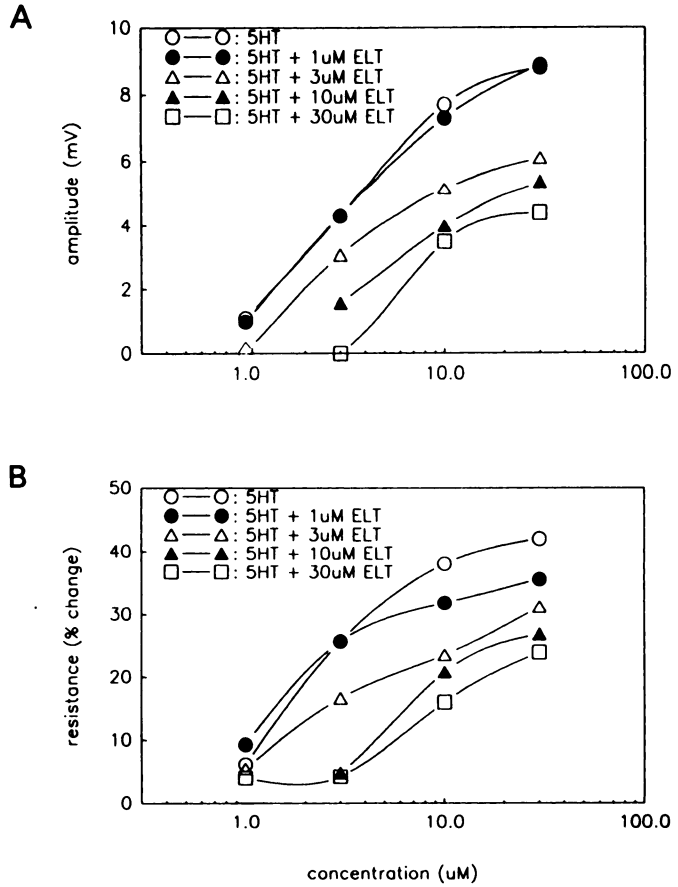

Fig. 5. Dose-response curves for the membrane hyperpolarization (A) and change in resistance (B) induced by serotonin under control conditions (n = 49), and in the presence of 1 (n = 7), 3 (n = 7), 10 (n = 14) or 30 (n = 7) μM eltoprazine. The symbols represent the average of all observations. Except for responses obtained with 1 μM and 3 μM eltoprazine on 1 μM 5HT, averaged responses established in the presence of the antagonist were significantly ($P < .05$) lower than the corresponding control responses to serotonin.

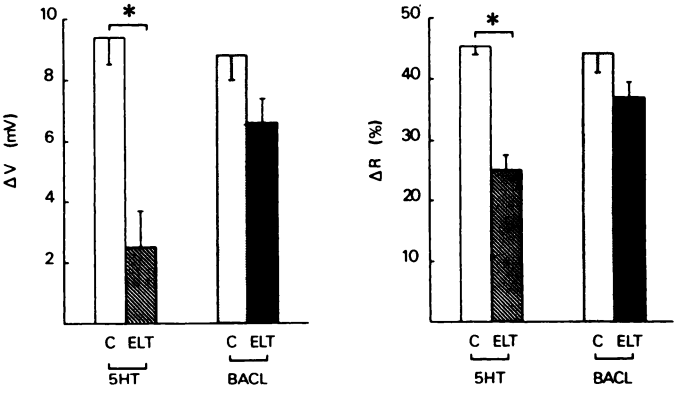

Fig. 6. The effects of 10 μM eltoprazine (ELT) on membrane hyperpolarization and changes in resistance evoked by 10 μM 5HT or 10 μM baclofen (BACL) in the same group of neurons (n = 4). Open bars, response to 5HT: light, stippled bar, response to BACL; dashed bars, response to 5HT in the presence of eltoprazine; dark bars, response to BACL in the presence of eltoprazine. All bars represent the mean ± S.E.M.; the data were tested with a Student's t test (*$P < .01$).

izing the membrane. We therefore compared the action of eltoprazine on 5HT- and baclofen-evoked membrane changes (fig. 6). While eltoprazine in these neurons suppressed the actions of 5HT ($P < .01$), it induced no significant reduction

of baclofen-induced changes in membrane potential and resistance.

Discussion

The aim of this study was to investigate the mechanism of action of eltoprozine on presumed $5HT_{1A}$ receptors with electrophysiological recording techniques. Previous studies in limbic structures have shown that the frequently observed membrane hyperpolarization in response to 5HT can be mimicked by compounds selective for the $5HT_{1A}$ receptors, e.g., 8OHDPAT, buspirone and ipsapirone (Andrade and Nicoll, 1987a,b; Joëls *et al.*, 1987; Wu *et al.*, 1988) and blocked by spiperone (Andrade and Nicoll, 1987a). In the present investigation we therefore used the membrane hyperpolarization induced by 5HT in hippocampal CA1 neurons as a test model for $5HT_{1A}$-mediated activity. We compared eltoprazine activity with 5HT rather than with any of the selective agonists, since the latter also display some antagonistic actions and, in addition, have long-lasting effects (Colino and Halliwell, 1986; Andrade and Nicoll, 1987b, Joëls *et al.*, 1987).

We have shown that in CA1 neurons eltoprazine, like 5HT, induces a membrane hyperpolarization accompanied by a reduction in input resistance, although the effects of eltoprazine are small compared to those of 5HT. The hyperpolarizing responses to eltoprazine are probably due to an increase in K^+ conductance, since the reversal potential for the eltoprazine effect was close to E_k and the hyperpolarizing responses remained stable when the recording pipette was filled with 3 M KCl. Changes in I_c and the delayed rectifier, both sensitive to TEA (Castle *et al.*, 1989), are unlikely to contribute to the action of eltoprazine, as eltoprazine-responses were unaffected by TEA. There are several indications that the observed action of eltoprazine is mediated by a $5HT_1$ receptor:

1. Binding studies have revealed that eltoprazine binds with high affinity to $5HT_{1A}$ (K_i = 37 nM), $5HT_{1B}$ (K_i = 59 nM) and $5HT_{1C}$ (K_i = 80 nM) sites (Schipper *et al.*, 1987). The affinity for non-$5HT_1$ receptors is much weaker, although a moderate affinity was found for *beta* adrenergic receptors (K_i = 200 nM). Yet, it is unlikely that *beta* receptors contribute to our eltoprazine effects, since *beta* agonists induce depolarization rather than hyperpolarization of the CA1 cell membranes (Madison and Nicoll, 1986a,b).

2. Similarly, it is unlikely that the eltoprazine effects are indirectly evoked by the release of other monoamines. As eltoprazine responses were still observed in the presence of 1 μM TTX, which was sufficient to abolish the synaptic response, eltoprazine probably acts directly on the postsynaptic membrane. Moreover, addition of antagonists for dopamine and *alpha* receptors, the only monoamine receptors mediating hyperpolarizing responses in the CA1 area (except for 5HT), did not interfere with the action of eltoprazine on membrane resistance.

3. Both the eltoprazine- and 5HT-induced hyperpolarizations were largely blocked by spiperone, a nonselective $5HT_{1A}$-antagonist.

In contrast to the agonistic actions of eltoprazine, the antagonistic actions of the drug appear to be quite pronounced. The antagonistic effects were rather prolonged, which is consistent with the lipophilic nature of the compound. In the presence of eltoprazine, dose-response curves for 5HT were shifted to the right with little effect on the slope. In addition, the results indicate that the maximal 5HT response was suppressed by eltoprazine. As eltoprazine itself displayed a low intrinsic activity we tested whether the antagonizing activity could be explained by shunting of the K^+ conductances. Therefore, we compared the action of eltoprazine on 5HT- and baclofen-induced responses since these responses are probably due to activation of the same K^+ conductance (Andrade *et al.*, 1986). Baclofen-induced responses were not significantly affected by eltoprazine, indicating that most of the antagonistic activity of eltoprazine is probably not due to shunting of the membrane. Clearly, the molecular mechanisms of the antagonistic properties of eltoprazine remains to be solved.

The potent antagonistic properties of eltoprazine on the presumed $5HT_{1A}$ receptor are of particular interest since no selective and potent $5HT_1$ antagonist is currently available. Thus, 5HT analogues with high affinity for the $5HT_{1A}$ site, *e.g.*, 8OHDPAT, buspirone and ipsapirone, do suppress 5HT responses partly, but have more pronounced agonistic effects (Colino and Halliwell, 1986; Andrade and Nicoll, 1987a,b; Joëls *et al.*, 1987). Conversely, compounds that fully suppress 5HT-induced hyperpolarizations, such as spiperone (Andrade *et al.*, 1986; Andrade and Nicoll, 1987a), are not selective for the $5HT_1$ receptors. The facts that i) eltoprazine displays a high binding affinity for $5HT_1$ sites, but not other receptors in the brain and ii) eltoprazine blocks 5HT-induced but not baclofen-induced hyperpolarizations or 5HT-evoked depolarizing responses in the CA1 hippocampal area suggests that eltoprazine might selectively affect 5HT-mediated effects in the hippocampus.

The here described antagonistic properties of eltoprazine were observed in the rat hippocampus, where $5HT_{1A}$ receptors are located postsynaptically (Gozlan *et al.*, 1983). In the brainstem, however, $5HT_{1A}$ receptors are probably not exclusively located at the postsynaptic site (Hall *et al.*, 1985). It is therefore possible that eltoprazine affects neurons in the brainstem in a different way than in the hippocampus, as was previously observed for ipsapirone (Andrade and Nicoll, 1987b; Sprouse and Aghajanian, 1987). In this respect, observations by Evans *et al.* (1988) with extracellularly recorded brainstem neurons are of interest. In this study, eltoprazine displayed agonistic rather than antagonistic activity on $5HT_{1A}$ sites.

In behavioral tests eltoprazine displays specific anti-aggressive activity (Olivier *et al.*, 1987). It was assumed that the anti-aggressive activity is linked to the action of eltoprazine on $5HT_{1B}$ receptors. We here show that eltoprazine also induces effects that are probably mediated by $5HT_{1A}$ sites. The presently demonstrated activity of eltoprazine might therefore also be important for the determination of the overall behavioral profile of the compound. In addition, eltoprazine could prove to be useful tool in electrophysiological studies to distinguish between $5HT_1$ or non-$5HT_1$ receptor-mediated activity.

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