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Effects of antidepressants on the inward current mediated by 5-HT_3 receptors in rat nodose ganglion neurones

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1 Effects of three different categories of antidepressants, imipramine (tricyclic), fluoxetine (selective 5-hydroxytryptamine (5-HT) uptake inhibitor), phenelzine and iproniazid (monoamine oxidase (MAO) inhibitor) on the inward current mediated by 5-HT_3 receptors were investigated in rat nodose ganglion neurones. The whole-cell patch-clamp technique was used for recording the 5-HT current.

2 All the antidepressants tested inhibited the peak 5-HT current. The inhibition gradually reached a steady level and the recovery was incomplete when antidepressants were removed. IC_{50} values for imipramine, fluoxetine and phenelzine were 0.54 μM, 1.3 μM and 4.2 μM respectively. The correspondent Hill coefficients were 0.9, 0.87 and 0.92.

3 The antidepressants examined increased the rate of 5-HT current desensitization. IC_{50} values for imipramine, fluoxetine and phenelzine on the decrease in desensitization time constant were 0.11 μM, 0.18 μM and 2.4 μM respectively. The correspondent Hill coefficients were 0.9, 1.14 and 1.06.

4 Intracellular applications of the protein kinase inhibitor, H-7 (100 μM), GDP-β-S (2 mM) and the calcium chelator BAPTA (20 mM) did not affect the 5-HT current and the actions of antidepressants on 5-HT current.

5 These results suggest that the 5-HT_3 receptor is an acting site for the therapeutic use of antidepressants. The present observation is also helpful in explaining the analgesic effect of antidepressants seen in pain clinics.

Keywords: 5-HT_3 receptor; desensitization; patch clamp; nodose ganglion neurone; imipramine; fluoxetine; phenelzine

Introduction

The 5-HT_3 receptor mediates fast synaptic transmission in the central nervous system (Sugita *et al.*, 1992). There is evidence that 5-HT_3 receptors and possibly the 5-HT_3 receptor-mediated synaptic transmission are involved in some psychiatric disorders such as depression (Fozard, 1992; Greenshaw, 1993). Behavioural studies have shown that 5-HT_3 receptor antagonists have a similar effect of that of antidepressants in an animal model of depression (Martin *et al.*, 1992). Receptor binding studies demonstrated a nanomolar binding of antidepressants to quipazine-labelled 5-HT_3 receptor sites in rat cortical membranes (Schmidt & Peroutka, 1989), but this observation was not confirmed by a study using the ICS205930-labelled 5-HT_3 receptor binding sites in neuroblastoma N1E-115 cells and zacopride-labelled binding sites in rat entorhinal cortex (Hoyer *et al.*, 1989). On the other hand, antidepressants are well known to block the 5-hydroxytryptamine (5-HT) reuptake pump and affect 5-hydroxytryptamine neurotransmission. Tricyclic antidepressants block the uptake of both noradrenaline and 5-HT and the antidepressant of the second-generation, fluoxetine, is a highly specific 5-HT uptake inhibitor (Fuller *et al.*, 1991; Hollister, 1992). At concentrations that inhibit the process of amine reuptake, antidepressants have no significant effect on neurotransmitter receptors investigated so far (Richelson & Nelson, 1984; Wander *et al.*, 1986). Antidepressants are also known to produce analgesia in pain clinics and to be especially useful for treating a variety of chronic pain (Hollister, 1992), while 5-HT_3 receptors have been shown to mediate various forms of pain such as the pain perception in peripheral, migraine, angina and irritable bowel syndrome (Fozard & Kalkman, 1992; Greenshaw, 1993). In the present study, the effects of three categories of widely used antidepressants on the inward current mediated by 5-HT_3 receptors in rat nodose ganglion neurones were investigated. They were: imipramine (tricyclic), fluoxetine (the selective 5-HT reuptake inhibitor), phenelzine and iproniazid (monoamine oxidase (MAO) inhibitor).

Methods

Preparation of nodose ganglion neurones

Single neurones were isolated from rat nodose ganglion. The procedure used has been described previously (Ikeda *et al.*, 1986) although some minor changes were made. Briefly, male, adult Sprague-Dawley rats (150–300 g) were killed by decapitation; nodose ganglia were rapidly dissected and placed in cold Dulbecco's modified Eagle's medium (DMEM). Nodose ganglia were then minced with iridectomy scissors and placed in DMEM containing 1.25 mg ml^{-1} collagenase (Sigma type IA), 0.8 mg ml^{-1} trypsin (Sigma type III) and 0.125 mg ml^{-1} deoxyribonuclease (Sigma type IV). The minced tissue was then digested in a waterbath shaker at 35°C for 30 to 45 min, after which soybean trypsin inhibitor (Sigma type IIs, 1 mg ml^{-1}) was added. Neurones were then plated in petri dishes.

Whole cell patch-clamp recording

Neurones were viewed with an inverted microscope and superfused with extracellular solution at 1 ml min^{-1}. The extracellular solution contained (in mM): NaCl 150, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, HEPES 10, D-glucose 10; the pH was adjusted to 7.4 with NaOH, and sucrose was added to adjust the osmolality to 340 mmol kg^{-1}. Experiments were performed at room temperature. The whole cell version of the patch-clamp technique was used by means of an Axopatch-1D amplifier (Axon Instruments). Patch electrodes (2–5 Mohm) were pulled from borosilicate glass (World Precision Instrument Co.) and filled with an internal solution containing (in mM): KCl 140, $MgCl_2$ 2, $CaCl_2$ 1, HEPES 10, EGTA 11, ATP (magnesium salt) 2; the pH was adjusted to 7.4 with KOH and osmolality to 310 mmol kg^{-1} with sucrose. Neurotransmitters and other drugs were dissolved in external solution and applied through a fast perfusion system consisting of a series of fused silica tubes (200–300 μm) glued together and held by a micromanipulator (Narishige). These tubes

were connected to several different reservoirs containing either control or test solutions. The neurone under study was placed within 50 μm of the opening of these tubes and the solution was allowed to perfuse the cell. By rapidly moving the perfusion system laterally, a different solution was applied to the cell. Ion substitution experiment shows that solution exchange is completed within 60 ms (Fan *et al.*, unpublished observations).

Values in the text and figures are means ± s.e.mean. Data were statistically compared by variance analysis or paired *t* test. Concentration-response curves were fitted with the logistic equation (De Lean *et al.*, 1978). Current decay was fitted by the use of Clampfit (Axon Instruments, Inc.). Fits were considered to be good if *r* values were >0.97.

5-HT hydrochloride, iproniazid and H-7 (1-(5 isoquinolinesulphonyl)-2-methyl-piperazine) were purchased from Research Biochemicals Inc. Imipramine, phenelzine and BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid) were from Sigma. GDP-β-S (guanosine-5′-O-(2-thiodiphosphate)) was from Boehringer Mannheim. Fluoxetine hydrochloride was a generous gift from Eli Lilly and Company.

Results

In acutely isolated rat nodose ganglion neurones, 5-HT produced an inward current mediated by 5-HT_3 receptors (Lovinger & White, 1991; Fan *et al.*, 1992). Usually, a stable 5-HT current can be observed 30 to 40 min after establishing whole cell recording conditions. The antidepressants tested (imipramine, fluoxetine, phenelzine and iproniazid) had similar effects on the 5-HT current. The effects of imipramine, fluoxetine and phenelzine were investigated in detail. A current was induced by 3 μM 5-HT which is close to the EC_{50} value of 2.6 μM in rat nodose neurones (Fan and Weight, unpublished data).

Effect of antidepressants on the peak 5-HT current

Data in Figure 1 show that a 3–4 min application of antidepressants inhibited the peak 5-HT current. After the introduction of antidepressants, the 5-HT current was gradually inhibited and the inhibition reached a steady level thereafter (Figure 4a). The recovery of the 5-HT current from the effects of antidepressants was incomplete (Figures 1 and 4a). Concentration-response curves of antidepressants are shown in Figure 2. IC_{50} values for imipramine, fluoxetine and phenelzine were 0.54 μM, 1.3 μM and 4.2 μM. The correspondent Hill coefficients were 0.9, 0.87 and 0.92.

Effect of antidepressants on the desensitization of the 5-HT current

Desensitization of the 5-HT current in the present study was best fitted by a single exponential function (fitting not shown). Antidepressants increased the rate of 5-HT current desensitization by reducing the desensitization time constant (Figures 1 and 3). Figure 3b shows superimposed currents induced by 3 μM 5-HT in control and in the presence of 1 μM fluoxetine. Concentration-response curves of the antidepressant effect on desensitization time constant are shown in Figure 3a. IC_{50} values for imipramine, fluoxetine and phenelzine were 0.11 μM, 0.18 μM and 2.4 μM respectively. The corresponding Hill coefficients were 0.9, 1.14 and 1.06. Similar to their effects on peak current, the reduction of 5-HT current desensitization time constant by antidepressants gradually reached a steady state level and the recovery was incomplete (Figure 4b).

Intracellular messengers in the actions of antidepressants on 5-HT current

There is evidence that intracellular messengers may influence the 5-HT_3 receptor desensitization (Yakel & Jackson, 1988; Yakel *et al.*, 1991). The slow effect of antidepressants on the 5-HT current and the incomplete recovery appear to be the characteristics of the second messenger-mediated actions. Therefore, effects of antidepressants on 5-HT current were investigated in the presence of the protein kinase inhibitor H-7, GDP-β-S, and calcium chelator, BAPTA. Intracellular applications of H-7 (100 μM), GDP-β-S (2 mM) and BAPTA (20 mM with 0 mM intracellular Ca^{2+}) for 10 to 15 min had no effect on the 5-HT current and the actions of antidepressants on 5-HT current (paired *t* test, $P>0.1-0.2$, Figure 5).

Discussion

Results in the present experiments demonstrate that the widely used antidepressants inhibit the peak 5-HT current

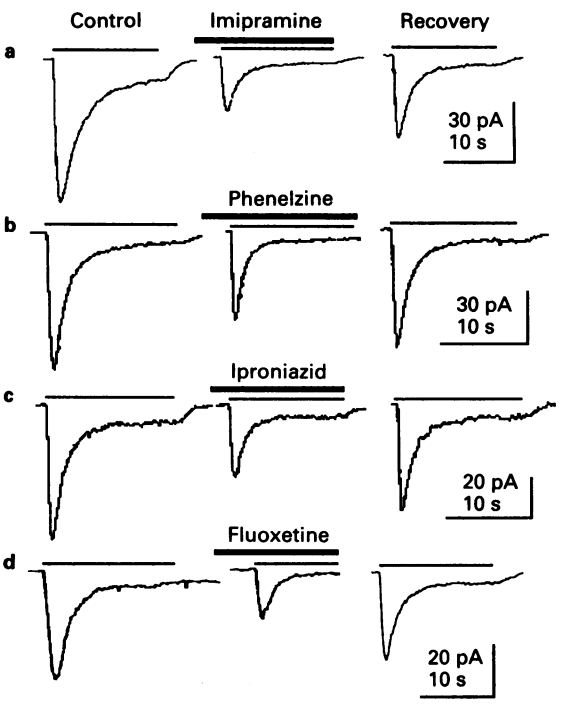

Figure 1 Effects of antidepressants on 5-hydroxytryptamine (5-HT) current. Currents were induced by 3 μM 5-HT which is indicated by thin bars. Recordings in (a), (b), (c) and (d) were from four different cells. Antidepressants (indicated by thick bars) were applied for 3–4 min and then applied together with 5-HT.

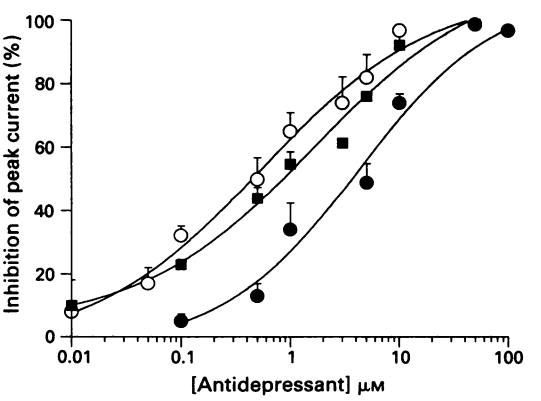

Figure 2 Concentration-response curves of antidepressants on the inhibition of peak current induced by 3 μM 5-hydroxytryptamine (5-HT). Each point represents the average data from 4–9 cells. Data were collected after 5 min application of the tested antidepressant. Standard errors are shown: (○) imipramine; (●) phenelzine; (■) fluoxetine.

and increase the rate of current desensitization. Their effects on 5-HT current were slow, concentration-dependent and the recovery was incomplete. Desensitization of the 5-HT current is obviously more sensitive to antidepressants than the peak current. IC_{50} values of imipramine, fluoxetine and phenelzine on current desensitization are 0.11 μM, 0.18 μM and 2.4 μM while those on peak current are 0.54 μM, 1.6 μM and 4.2 μM. It is possible that the increased desensitization contributes to the inhibition of peak current by antidepressants. Since the effects of antidepressants on 5-HT current were not affected by H-7, GDP-β-S and calcium chelator BAPTA, it is unlikely that G-protein, protein kinases and calcium-dependent processes are involved in the action of these antidepressants.

The daily dosage of imipramine for the treatment of depression is 75–200 mg and those for fluoxetine and phenelzine are 20–80 mg and 45–75 mg respectively (Hollister, 1992). It has been reported that after a single dose of 40 mg fluoxetine, the patient's peak plasma fluoxetine concentration was from 0.042 μM to 0.16 μM and a steady-state plasma concentration of 0.26 to 0.88 μM of fluoxetine was found in patients after thirty-day administration of 40 mg per day (Goodnick, 1991). It has also been reported that the effective plasma concentration of imipramine is between 100 ng ml^{-1} to 300 ng ml^{-1} (0.36 μM to 1.07 μM, Hollister, 1992). Therefore, the effective concentrations of imipramine and fluoxetine observed in the present study are within their therapeutic concentrations. The effective plasma concentration of phenelzine is not well documented. Usually, desirable inhibition of monoamine oxidase can be achieved with a daily dosage of 1 mg kg^{-1} (Hollister, 1992) or 7.3 μM if it is completely absorbed, which is close to the IC_{50} values of phenelzine on 5-HT current (2.4 μM and 4.2 μM). These data suggest that the 5-HT$_3$ receptor is an acting site for the therapeutic use of antidepressants.

Fluoxetine has been shown to increase the synaptic poten-

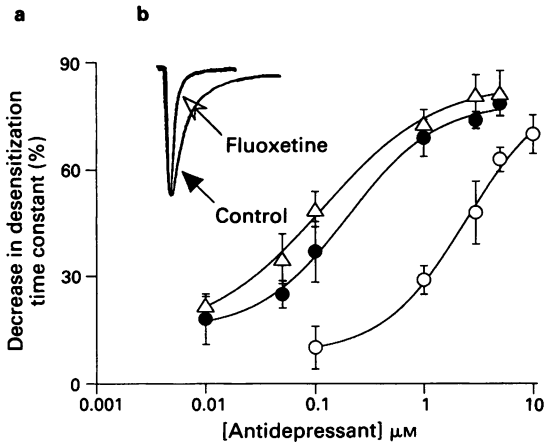

Figure 3 Effect of antidepressants on 5-hydroxytryptamine (5-HT) current desensitization. All currents were induced by 3 μM 5-HT. (a) Concentration-response curves of antidepressants on the reduction of desensitization time constant. Decreases in desensitization time constant were plotted against concentrations of antidepressants (in μM). Each point represents the average data from 3–9 cells. Data were collected after 5 min application of tested antidepressant. Standard errors are shown. (△) Imipramine; (○) phenelzine; (●) fluoxetine. (b) Sample recordings obtained in control (time constant: 1698 ms) and the presence of 5 μM fluoxetine (amplitude normalized to that of control, time constant: 355 ms).

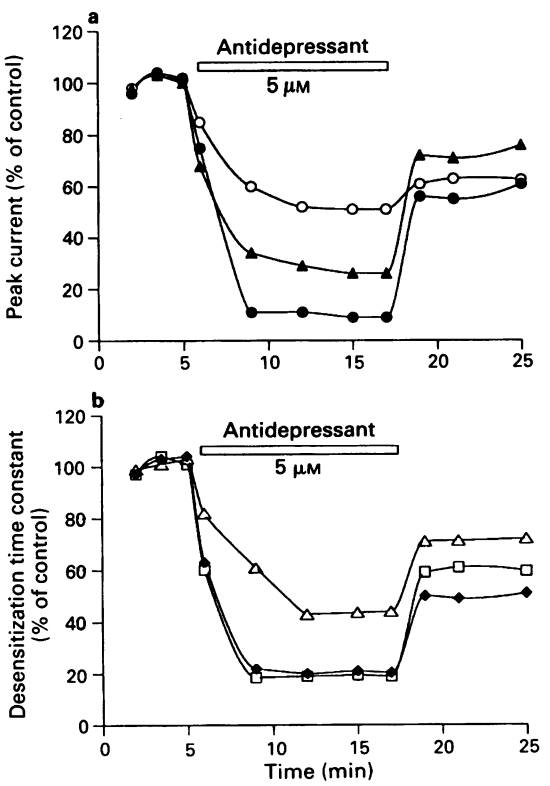

Figure 4 Time course of the effects of antidepressants on 5-hydroxytryptamine (5-HT) current. Currents were induced by 3 μM 5-HT. Three samples were collected before the administration of antidepressants and the average was taken as 100%. Compared to control, the difference for all the points after the application of 5 μM antidepressants (indicated by a bar) is highly significant ($P<0.05$–0.001), $n = 7$; 5, 9 for phenelzine, imipramine and fluoxetine respectively. (a) Effect on peak current: (●) imipramine; (○) phenelzine; (▲) fluoxetine. (b) Effect on current desensitization: (□) imipramine; (△) phenelzine; (◆) fluoxetine.

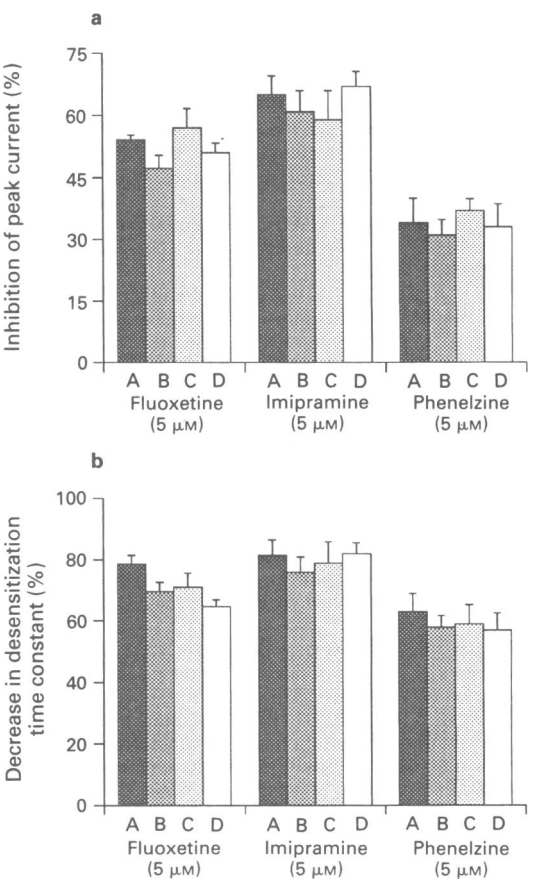

Figure 5 Effects of antidepressants on 5-hydroxytryptamine (5-HT) current under different conditions. Currents were induced by 3 μM 5-HT. Effects of 1 μM antidepressants were examined with and without intracellular H-7, GDP-β-S and BAPTA. Each category of experiment contains the average data from 4–7 cells. Standard errors are shown: (A) control; (B) H-7; (C) BAPTA; (D) GDP-β-S.

tials mediated by 5-HT_3 receptors in brain slices (Sugita *et al.*, 1992), while the present study demonstrates a 5-HT_3 receptor antagonist property of fluoxetine in isolated nodose ganglion neurones. There may be several reasons for the different effects of fluoxetine. First, it is likely that fluoxetine inhibited 5-HT uptake in brain slices and therefore increased the concentration of 5-HT in the synaptic cleft, resulting in increased synaptic potentials. In the present experiments, isolated nodose neurones were rapidly superfused and flooded with 5-HT when the agonist was applied. Thus, inhibition of 5-HT uptake by fluoxetine in this study should not have altered the concentration of 5-HT bathing the neurones. Second, stimulation of presynaptic terminals may activate both excitatory and inhibitory synaptic transmissions. Fluoxetine may inhibit the 5-HT_3 receptor-mediated excitation on inhibitory interneurones, block some of the inhibitory transmissions and therefore increase the excitatory synaptic potential. Third, 5-HT_3 receptors may have different pharmacological properties in different species and different tissues (Tyers, 1990; Newberry *et al.*, 1991; Peters *et al.*, 1992). The 5-HT_3 receptor in the central nervous system may be less sensitive or insensitive to fluoxetine. If this is the case, the antidepressant effect of fluoxetine is not mediated by its action on the peripheral 5-HT_3 receptors.

Antidepressants have been used in patients to produce pain relief (Hollister, 1992) and 5-HT_3 receptors have been reported to mediate the peripheral pain perception and the flare response induced by local application of 5-HT in man (Richardson *et al.*, 1985; Orwin & Fozard, 1986). 5-HT_3 receptor antagonists blocked these painful responses and reduced acute and chronic inflammatory pain (Eschalier *et al.*, 1989; Giordano & Rogers, 1989; Giordano & Dyche, 1989). 5-HT_3 receptor antagonists may also be effective in migraine, angina and irritable bowel syndrome (for review, see Fozard & Kalkman, 1992; Greenshaw, 1993). These observations suggest that the inhibition of 5-HT_3 receptors by antidepressants may help to explain the analgesic effect of antidepressants.

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