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Electrophysiological effects of fluoxetine and duloxetine in the dorsal raphe nucleus and hippocampus

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Abstract

The cellular electrophysiological effects of duloxetine (LY248686), a dual serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine reuptake inhibitor, and the selective serotonin reuptake inhibitor fluoxetine were compared on spontaneously active neurons in the dorsal raphe nucleus and the hippocampus of chloral hydrate-anesthetized male rat. Systemic intravenous administration of duloxetine or fluoxetine inhibited dorsal raphe nucleus cell firing in a dose-dependent manner; duloxetine suppressed cell firing at significantly lower doses (ED_{100} 1.4 ± 0.3 mg/kg) than fluoxetine (ED_{100} 10.0 ± 2.0 mg/kg). In the hippocampus, microiontophoretic application of duloxetine or fluoxetine (0.01 M, pH 5.5; 5–40 nA) produced minimal inhibition of cell firing. When duloxetine was co-applied with 5-HT, the recovery response (RT_{50} values) of hippocampal pyramidal neurons to 5-HT application was not altered. In contrast, co-application of fluoxetine with 5-HT at the same iontophoretic currents significantly increased (59%) the RT_{50} values produced by 5-HT application alone. This physiological and pharmacological study contributes to understanding the cellular mechanisms of these agents which may be useful in the treatment of depression.

Keywords: Duloxetine; Fluoxetine; Hippocampus; Raphe nucleus, dorsal; Electrophysiology, cellular

1. Introduction

Duloxetine (LY248686), a recently developed reuptake blocker, demonstrates selectivity for both the serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine transporter (Engleman et al., 1995; Kasamo et al., 1996; Wong et al., 1993). These actions of duloxetine on biogenic amines have been confirmed by behavioral and neurochemical studies. For example, duloxetine is as effective as tricyclic antidepressants in attenuating immobility in a forced swim test (Katoh et al., 1995). Following duloxetine administration, 5-HT and norepinephrine accumulation increases in synaptosomal (Wong et al., 1993) and hippocampal slice preparations (Kasamo et al., 1996). Oral administration of duloxetine produces a dose-dependent increase in 5-HT and norepinephrine release in the rat frontal cortex and nucleus accumbens (Kihara and Ikeda, 1995). Additionally, intraperitoneal administration of duloxetine antagonizes the depletion of 5-HT and norepinephrine produced by pretreatment with *p*-chloroamphetamine or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, respectively (Fuller et al., 1994).

Therapeutic regimens which involve co-administration of antidepressants selective for the serotonergic or noradrenergic neurotransmitter systems may be more effective than treatment with either antidepressant alone (Nelson et al., 1991; Seth et al., 1992). Such results suggest that duloxetine may be a more effective treatment for depression than the use of a selective serotonin reuptake inhibitor, such as fluoxetine. However, cellular electrophysiological studies which directly compare duloxetine to the more thoroughly characterized selective serotonin reuptake inhibitors are limited. Focusing on the serotonergic properties of duloxetine, the present study compared the physiological and pharmacological characteristics of this compound to fluoxetine in the hippocampus and dorsal raphe nucleus.

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2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (Charles River, Wilmington, DE, USA; 250–400 g) were group housed with a 12 h light/dark cycle (lights on 07:00 h) under standard conditions. Food and water were available ad libitum.

2.2. Drug solutions

Duloxetine HCl (0.01 M in 0.1 M NaCl; courtesy of Eli Lilly, Indianapolis, IN, USA), fluoxetine (0.01 M; Sigma, St. Louis, MO, USA) and serotonin creatinine sulfate (0.04 M; Research Biochemicals International, Natick, MA, USA) were prepared for microiontophoresis (pH 5.5). For systemic intravenous studies, duloxetine and fluoxetine (2.5 mg/ml) were dissolved in saline (0.9% NaCl). All drug solutions were aliquoted and used fresh or stored at −80°C until use.

2.3. In vivo extracellular recordings

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.; Research Biochemicals International) and placed in a Kopf small animal stereotaxic instrument. A lateral tail vein was cannulated and used to maintain a constant level of anesthesia throughout the experiment. Body temperature was maintained at approximately 36°C using a thermostatically regulated heating pad. Conventional extracellular recording techniques were used to amplify and display waveforms using a window discriminator (WDR 420, Fintronics, Orange, CT, USA) as previously described (Cunningham and Lakoski, 1990).

Extracellular recordings in the dorsal raphe nucleus were carried out according to Cunningham and Lakoski (1990). For systemic drug administration, a baseline firing rate was established over several minutes and test drugs were then administered in 2-min intervals via the cannulated tail vein in a cumulative dose manner until cell firing was completely suppressed. The percent inhibition of cell firing (% inhibition) was calculated by comparison of the mean baseline firing rate obtained from a 1 min interval prior to the initial drug application and the mean cell firing rate obtained from a 1-min interval following each drug infusion. ED_{50} and ED_{100} values were determined for each compound from the cumulative dose-response curves fit with a linear regression (Sigma Stat, Version 1.0; Jandel Scientific, San Rafael, CA, USA). Only data obtained from histologically confirmed dorsal raphe nucleus neurons were included for further analysis with only one cell per animal tested for a drug-induced response.

Extracellular recordings from pyramidal neurons of the CA1 and CA3 regions of the hippocampus were conducted according to procedures of Dugar and Lakoski (1997). The percent inhibition of cell firing produced by drug application was determined by comparison of the mean baseline firing rate obtained from a 1-min interval prior to drug application and the mean cell firing rate obtained during a 1-min interval of drug application. Inhibition of pyramidal cell firing produced by drug application was assessed by calculating an IT_{50} value (nC) as defined by De Montigny and Aghajanian (1977) (current (nA) × time (s) to produce a 50% decrease in firing rate). The RT_{50} value was determined (time (s) to recover to 50% of the mean baseline firing rate) during the minute immediately following agonist application (De Montigny et al., 1980). Only data obtained from histologically identified neurons in the CA1 or CA3 hippocampal subfields were included for further analysis; typically, no more than two cells per animal were included for analysis.

2.4. Statistical analysis

Results are reported as mean ± standard error of the mean (S.E.M.). Statistical comparisons were made using a one-way analysis of variance (ANOVA) across all groups with the Student-Neuman-Keuls method for pairwise comparisons with a $P < 0.05$ level of significance (Sigma Stat, Version 1.0; Jandel Scientific, San Rafael, CA, USA). Data collected from CA1 and CA3 hippocampal neurons were combined due to a lack of significant difference in parameters recorded.

3. Results

3.1. Intravenous administration of duloxetine or fluoxetine in the dorsal raphe nucleus

Intravenous administration of duloxetine inhibited spontaneously active dorsal raphe neurons (Fig. 1). Duloxetine rapidly produced a complete inhibition of cell firing (Fig. 1, top panel). Similarly, fluoxetine produced complete inhibition of cell firing (ED_{50} and ED_{100} values 5.2 ± 1.2 and 10.0 ± 2.0 mg/kg, respectively; $n = 4$; data not shown); however, the cumulative dose of fluoxetine was significantly greater than observed with duloxetine administration (0.7 ± 0.2 and 1.4 ± 0.3 mg/kg, respectively; $P < 0.05$; $n = 6$). Once cell firing was completely suppressed, recovery to original baseline rates was rarely observed within 15 min of termination of duloxetine or fluoxetine administration.

3.2. Microiontophoretic application of duloxetine or fluoxetine in the hippocampus

The effects of duloxetine and fluoxetine on spontaneous cell firing of CA1 and CA3 pyramidal neurons in the hippocampus were compared. Direct application of duloxetine or fluoxetine utilizing a range of currents (5–40 nA) produced a maximal 20–30% inhibition of cell firing; no

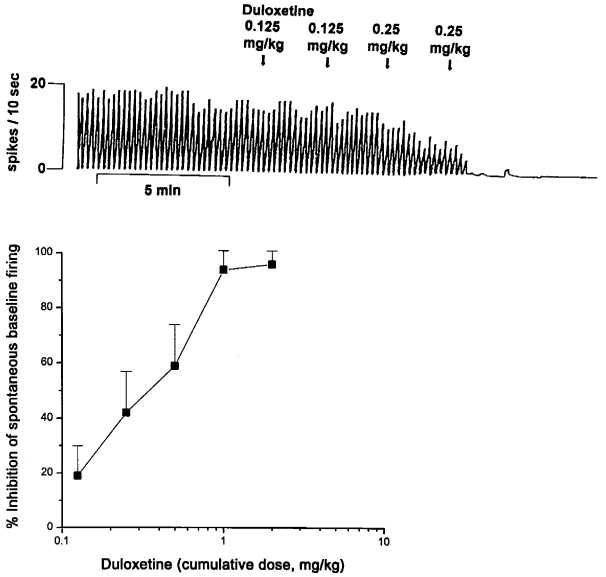

Fig. 1. Effect of duloxetine administered intravenously in a cumulative dose-response manner on spontaneously active dorsal raphe neurons recorded in chloral hydrate-anesthetized male rats. Top panel: A representative integrated firing rate histogram of dorsal raphe neurons illustrates the inhibition of cell firing produced by the acute i.v. administration of duloxetine. Duloxetine rapidly produced 50% inhibition of spontaneous cell firing (cumulative dose = 0.5 mg/kg, i.v.). No recovery of spontaneous cell firing was noted. Bottom panel: The dose-response curve for the inhibition of cell firing produced by i.v. administration of duloxetine in the dorsal raphe nucleus ($n = 6$). Only one cell per animal was recorded and tested for sensitivity to duloxetine.

cell tested reached 50% inhibition ($n = 34$; data not shown). Currents greater than 40 nA produced electrode blockage. Prolonged application of duloxetine or fluoxetine (5–10 min) at 10 nA failed to significantly alter baseline firing rates. However, in a few cells ($n = 8$) a gradual decrease in baseline firing rate was noted during prolonged applications of duloxetine or fluoxetine (Fig. 2).

As illustrated in the representative frequency histograms of hippocampal pyramidal cell firing, co-application of duloxetine (10 nA) and 5-HT (20 nA) did not alter the inhibitory response (IT_{50} values) produced by the application of 5-HT alone (Fig. 2, top panel). Furthermore, the time to recovery (RT_{50} values) was not altered by duloxetine co-application with 5-HT. Similar to duloxetine, co-application of fluoxetine (10 nA) with 5-HT did not potentiate the inhibitory response to 5-HT (10 nA), i.e., the IT_{50} values were not consistently decreased (Fig. 2, middle panel). However, in contrast to duloxetine, fluoxetine co-applied with 5-HT increased the time to recovery following 5-HT application alone.

The effects of duloxetine and fluoxetine co-application on the RT_{50} values produced by 5-HT application alone are summarized in Fig. 2, bottom panel. Duloxetine co-applied with 5-HT did not alter the recovery response of hippocampal pyramidal neurons to the application of 5-HT alone ($n = 14$ cells). In contrast, co-application of fluoxetine significantly increased (59%; $P < 0.05$) the time to recovery of baseline firing rates following 5-HT application ($n = 11$ cells).

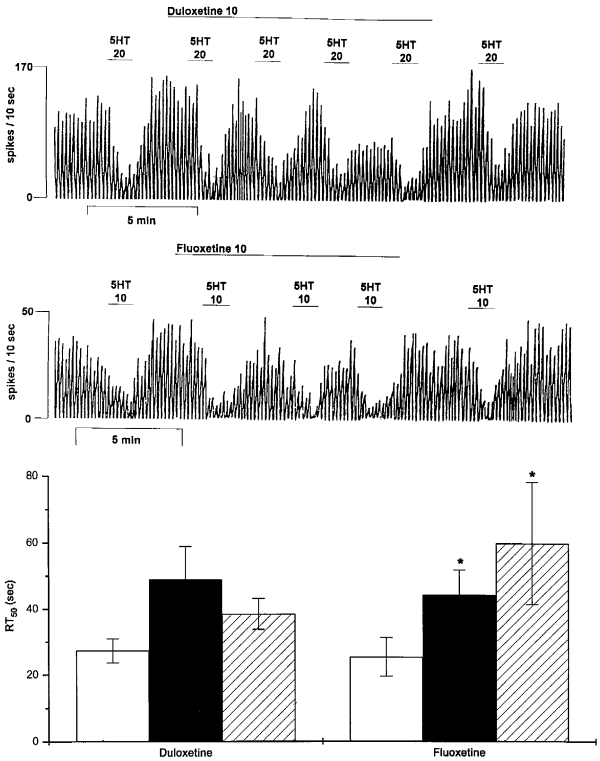

Fig. 2. Effects of microiontophoretic application of duloxetine and fluoxetine on the 5-HT inhibitory and recovery responses recorded in spontaneously active hippocampal pyramidal neurons in the chloral hydrate-anesthetized male rat. Top panel: In this representative integrated firing rate histogram of hippocampal pyramidal cell firing, 5-HT applied alone at 20 nA inhibited neuronal firing by 50% ($IT_{50} = 600$ nC) with a rapid recovery ($RT_{50} = 20$ s). Co-application of duloxetine (10 nA) did not significantly increase the recovery response to 5-HT ($RT_{50} = 30$, 30, 20 and 20 s; first, second, third and fourth co-application, respectively). Similarly, duloxetine co-application did not change the IT_{50} values produced by application of 5-HT ($IT_{50} = 600$, 800, 600 and 800 nC; first, second, third and fourth co-application, respectively). Middle panel: Serotonin applied at 10 nA inhibited neuronal firing by 50% ($IT_{50} = 600$ nC) with a rapid recovery response ($RT_{50} = 10$ s). Co-application of fluoxetine (10 nA) prolonged the recovery phase to the application of 5-HT ($RT_{50} = 50$, 30 and 50 s; first, second and third co-application, respectively). Similar to duloxetine, fluoxetine co-application did not consistently alter the IT_{50} values ($IT_{50} = 200$, 500 and 200 nC; first, second and third co-application, respectively). Following fluoxetine co-application, the recovery response returned to the baseline value ($RT_{50} =$ 10 s). Bars indicate the duration of drug application (min) with current given in nA. Bottom panel: As compared to the application of 5-HT alone, the RT_{50} values for the first and second co-application of duloxetine and 5-HT did not change ($n = 14$). In contrast, fluoxetine co-application significantly increased the time to recovery from inhibition produced by application of 5-HT alone (44% and 59%; first and second co-applications of fluoxetine and 5-HT, respectively; $n = 11$). White bars indicate values for the application of 5-HT alone, black and hatched bars indicate values for the first and second co-applications of 5-HT and duloxetine or fluoxetine, respectively. * Significance from the application of 5-HT alone in the fluoxetine group ($P < 0.05$).

4. Discussion

Systemic administration of fluoxetine has been shown to increase the synthesis of 5-HT (Tsuiki et al., 1995) and the levels of 5-HT in the hippocampus and dorsal raphe nucleus (Fuller et al., 1994; Guan and McBride, 1988; Kreiss and Lucki, 1995; Malagié et al., 1995). In clinical settings, fluoxetine demonstrates an enhanced efficacy in the treatment of depression over placebo (Stark and Hardison, 1985) and produces fewer side effects than tricyclic antidepressants (Rickels and Schweizer, 1990; Stark and Hardison, 1985). With similar serotonergic properties as fluoxetine, the physiological properties of duloxetine have yet to be fully characterized.

In the present study, systemic administration of duloxetine and fluoxetine suppressed spontaneous cell firing in the dorsal raphe nucleus. The inhibitory actions of fluoxetine on cell firing in this brain region are in agreement with a previous report from our laboratory utilizing fluoxetine (Cunningham and Lakoski, 1990). Systemic administration of duloxetine suppressed dorsal raphe nucleus cell firing in a dose-dependent manner, however, at significantly lower doses than fluoxetine. While duloxetine has been previously shown to inhibit serotonergic neuronal firing (Kasamo et al., 1996), the reported ED_{50} value was lower than obtained in the present study; this difference in inhibitory potency may be due to injection protocols (bolus versus dose response) or vehicle solutions utilized. As found in the present study, the difference in sensitivity to systemically administered fluoxetine versus duloxetine may, likewise, be due to intrinsic differences in the transport of these compounds across the blood-brain barrier and/or potency for 5-HT reuptake (IC_{50} values $= 2.6 \pm 0.4$ nmol/l and 22.4 ± 3.6 nmol/l, duloxetine and fluoxetine, respectively; Wong et al., 1993).

Using microiontophoretic application, the effects of duloxetine and fluoxetine applied alone and co-applied with 5-HT on hippocampal pyramidal cell firing were evaluated. The inhibitory effects of duloxetine or fluoxetine applied alone to hippocampal neurons were minimal and IT_{50} values for 5-HT were not altered by the co-application of either duloxetine or fluoxetine. Co-application of fluoxetine significantly enhanced the recovery phase of hippocampal neurons following 5-HT application. However, duloxetine co-applied with 5-HT failed to alter the recovery response of pyramidal neurons produced by the application of 5-HT alone. The difference in 5-HT receptor binding profiles established for these drugs (Jenck et al., 1993; Hyttel, 1994) may underlie their different physiological profiles.

The characterization of fluoxetine as a selective serotonin reuptake inhibitor underscores the utility of this agent as a therapeutic treatment for depression. However, patients with resistant depression, individuals demonstrating minimal improvement following treatment with tricyclic antidepressants or selective serotonin reuptake inhibitors, have responded positively to combination therapy of these classes of antidepressants (Nelson et al., 1991; Seth et al., 1992). Therefore, duloxetine, a prototypical dual 5-HT and norepinephrine reuptake inhibitor, may be useful for the treatment of individuals with resistant depression.

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