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Selective Serotonin Reuptake Inhibitors May Enhance Responses to Noxious Stimulation

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DIRKSEN, R., E. L. J. M. VAN LUIJTELAAR AND C. M. VAN RIJN. *Selective serotonin reuptake inhibitors may enhance responses to noxious stimulation.* PHARMACOL BIOCHEM BEHAV **60**(3) 719–725, 1998.—The acute effects of various doses of two selective serotonin reuptake inhibitors (fluoxetine and fluvoxamine) on thermal and electrical stimulation-induced pain were investigated in drug-naive Wistar rats. The hot-plate and the tail-flick test and the noxious-induced withdrawal test were used. The two drugs had no effects on heat-induced pain behavior. However, the two compounds enhanced the motor responses induced by noxious electrical stimulation. These data contrast to what is generally found for tricyclic antidepressants and suggest a modality specific pain system. Cardiac and blood pressure were also found to change, but these changes were not correlated to changes in nociception. Taken together, the data suggest that the acutely administered selective serotonin reuptake inhibitors may exacerbate an acute type of pain. © 1998 Elsevier Science Inc.

Nociception Fluoxetine Fluvoxamine Noxious-induced withdrawal reflex

THERAPEUTIC effects of tricyclic antidepressant drugs in the treatment of pain have been reported extensively (8,34, 41,44,55). The analgesia by tricyclic antidepressants may rely on different mechanisms, as these drugs are known to be nonselective reuptake inhibitors. Thereby, antidepressant drugs exert their action (to a varying degree) on multiple targets that are also involved in the pain systems, such as the histaminergic, α-adrenergic, serotonergic, muscarineric, and NMDA receptors (9,11,23,25,28,33,45). Another possibility is that the analgesic effects in humans may involve antidepressant actions, and they might change the perception of pain by an alleviation of mood (26,42,47,59).

Today, selective uptake blockers are available. Fluoxetine was the first reuptake inhibitor demonstrated to have a high degree of selectivity in blocking the neuronal uptake of serotonin (2,3). Subsequently, the even more selective serotonin reuptake inhibitor fluvoxamine was introduced (7). Serotonin was proposed as an important link to tricyclic antidepressant action (52). Also, serotonergic pathways play a role in pain and analgesia as indicated by the ability to produce analgesia by perfusing the dorsal surface of spinal cord with serotonin and to depress nociceptive responses of neurons of the dorsal horn [e.g., (4,27,54)]. In rats, large serotonergic descending pathways from the nucleus raphe magnus to the spinal cord are described (6), and serotonin-induced analgesia was enhanced by fluoxetine (63).

Clinical testing of selective serotonin reuptake inhibitors (SSRIs) showed that these drugs may inhibit pain in some conditions (48), but generally analgesia could not be convincingly accomplished [e.g. (26,42,47,59)]. Single oral doses of fluvoxamine resulted in inhibition of subjective pain rating in human volunteers subjected to electrical stimuli, but this treatment did not result in inhibition of R-III reflex responses (10). Fluoxetine was without effect for the acute type of pain in the postoperative period, and the drug may antagonize the effects of morphine (26). So it seems that the analgesic effect of SSRIs is restricted to some conditions only. An indication that SSRIs modify the sensory transmission in a modality-dependent fashion was the finding that fluoxetine intrathecally, inhibits substance P-induced scratching, but the same reuptake inhibitor had no effect on the tail-flick response (29).

Recently, we found that the magnitude of the antinociceptive effect of nonselective reuptake inhibitor amitryptiline depended on the type of noxious stimulation. The electrically induced withdrawal reflex was inhibited at lower doses of amitryptiline, but also differentially affected, compared to

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heat stimulation (20). Furthermore, also from earlier work it became evident that the withdrawal reflex is extremely powerful for detecting analgesia induced changes (13–17,19, 20,60). The purpose of the present study is to investigate whether a relation between modality of pain and effect of SSRIs underlies the restricted analgesic effect of SSRIs. Therefore, we assessed whether fluoxetine and fluvoxamine can inhibit responses elicited in the three different tests for nociception.

METHOD

Experimental Animals

Drug-naive male outbred Wistar rats Cpb WU (CPB-TNO, Zeist, The Netherlands), with a body weight of 250–300 g, were used ($n = 132$). Preexperimental conditions were standardized. Five animals were housed together in a macrolon cage and received standard food and tap water ad lib. They were kept on a 12 L:12 D cycle, with white lights on at 0800 h; the environmental temperature was kept at 21°C.

Drug Injections

The drugs administered were fluoxetine·HCl (Lilly and Company, Indianapolis, IN) and fluvoxamine·maleate (Solvay Duphar, Weesp, The Netherlands). The solvent was glucose 5%. Solutes were freshly prepared shortly before administration. Dosages refer to the salt. Control experiments included the intravenous (IV) injections of glucose 5% in a volume of 1 ml·kg^{-1}. The intravenous injections were performed directly in a tail vein or through the intrajugular catheter. The dosages used were 0.5, 1, 2, and 4 mg·kg^{-1}; in addition, 0.25 mg·kg^{-1} was used in the test of NIWR (see below, $n = 12$ animals). The drugs were delivered in a volume of 1 ml·kg^{-1}. The catheter wash was 0.25 ml.

Tests for Nociception

The three tests of nociception were performed at an environmental temperature of 21°C.

Thermal algesic tests. The thermal algesic tests were the hot-plate and the tail-flick test. They were performed in this order in 60 rats (six rats per dose of each drug). The hot plate was maintained at 52.5°C. The interval was measured between the time the rat was placed on the hot plate and the time the rat started to lick a hind paw. The tail flick was evoked by placing the tail of the animals over a slit under which a halogen projection lamp (300 watt) was placed. Radiant heat was focussed on the ventral surface of the tail, approximately 2 cm from the tip, and the interval was measured between the time the light was switched on and the time when the tail was flicked away. In both tests, these intervals are known as the response latencies. If a response was absent, tissue damage was limited by an empirically assessed standard cutoff time of 60 s in the hot-plate test and 30 s in the tail-flick test. Response latencies were assessed prior to drug injection and 5, 10, 15, 30, 60, 90, 120, and 240 min after injection. The temperatures of the skin of a hind paw (at the dorsal surface) and the tail (at the dorsal surface at approximately 8 cm from where the beam was focused) were measured (using a noncontact infrared measurement adaptor Fluke 80T-IR; John Fluke Inc., Germany) prior to drug injection and prior to the measurement of response latencies (50).

Noxious-induced withdrawal reflexes (NIWRs). This procedure is described, evaluated and used extensively elsewhere [e.g. (17,20)]. In brief, rats were anesthetized with an intraperitoneal (IP) injection of urethane (1.2 g·kg^{-1}) to allow cannulation of the right internal jugular vein. Next, aliquots urethane (Riedel-de Haën AG, Seelze-Hannover, Germany) were injected intravenously to a total of 0.2 g·kg^{-1}, and the trachea and right carotid artery were cannulated. This total dose of urethane (1.4 g·kg^{-1}) delivered within the first half hour maintained anesthesia during the experiments. After cannulation, the rat was placed on the experimentation table. The trachea cannula was used for artificial normoventilation. The cannula in the carotid artery was connected to a pressure transducer (Viggo-Spectramed, BOC, USA), which provided a measure for the intraarterial (IA) pressures. The heart rate was derived from the arterial pressure signal (heart rate counter AT-601G, Nihon Kohden Corp., Japan). The right hind paw was mounted in a shoe that contained two electrodes allowing transcutaneous bipolar stimulation (Grass stimulator S11, with stimulus isolation unit SIU5A, and constant current unit CCU1A). Stimulation parameters were set to 4-ms pulse duration, 7.5-mA stimulus strength, 100-Hz pulse frequency, in a train of 500 ms duration, and a repetition rate of 12.5 mHz (0.75 min^{-1}) for the trains (i.e., every 80 s a stimulus). The hind paw was also connected to a force-displacement transducer (TB-611T, Nihon Kohden Corp., Japan), which allowed the measurement of the withdrawal force response to the electrical stimulus.

Measures

The hot-plate and tail-flick tests. The effect was defined as the maximum change within 30 min after injecting the drug. The maximum percentages of effect (MPE) after injection of the drug were compared with those after injection of glucose 5%.

The MPE for each animal was calculated as

$$\text{MPE} = \frac{\text{postinjection RL} - \text{preinjection RL}}{\text{cutoff time} - \text{preinjection RL}} \times 100$$

where the postinjection response latency (RL) is the maximum change in latency after injection of drug or glucose 5%. If the postinjection response latency was shorter than the preinjection response latency, then the preinjection response latency was used as denominator in the ratio of the equation.

NIWRs. The individual baseline withdrawal force (expressed in g) was measured over a 20-min period. The average of the baseline responses was calculated and served to determine the individual relative responses expressed as percentage of this mean baseline response (17).

The drug-induced effect is defined as the difference between the mean of the baseline responses (100%) and the response after injection of the drug. The time until return to baseline values served to compare the duration of the effects.

Statistical Analysis

The percentages of effect were analyzed with a one-way or two-way analysis of variance (ANOVA). Post hoc evaluation included fitting the data to the equations of linear regression, one phase exponential association, or a polynome. Subsequently, the best fit was used to describe dose–response relationships. A difference yielding a p-value of less than 0.05 was considered as being significant. Each data point is derived from measurements in a minimum of 6 rats, and is given as the mean and SEM.

RESULTS

Predrug Responses and Control Data

The predrug response latencies in the hot plate test were (19.2 ± 2.5 s), and those of the tail flick test (8.1 ± 1.1 s). The coefficients of variation were of similar magnitude for the hot-plate and tail-flick test (33 and 30%, respectively). The control data for force of withdrawal elicited by the electrical stimuli in the urethane anesthetized rats was 57 ± 0.4 gf. The pre-injection coefficient of variation of NIWR responses was 4.2%, and the average of the coefficients of variation after glucose was 8.7% during the 1-h period.

Control data for the cardiovascular variables were: heart rate 416 ± 3 bpm, systolic IA pressure 150 ± 1 mmHg, and diastolic IA pressure 80 ± 1 mmHg.

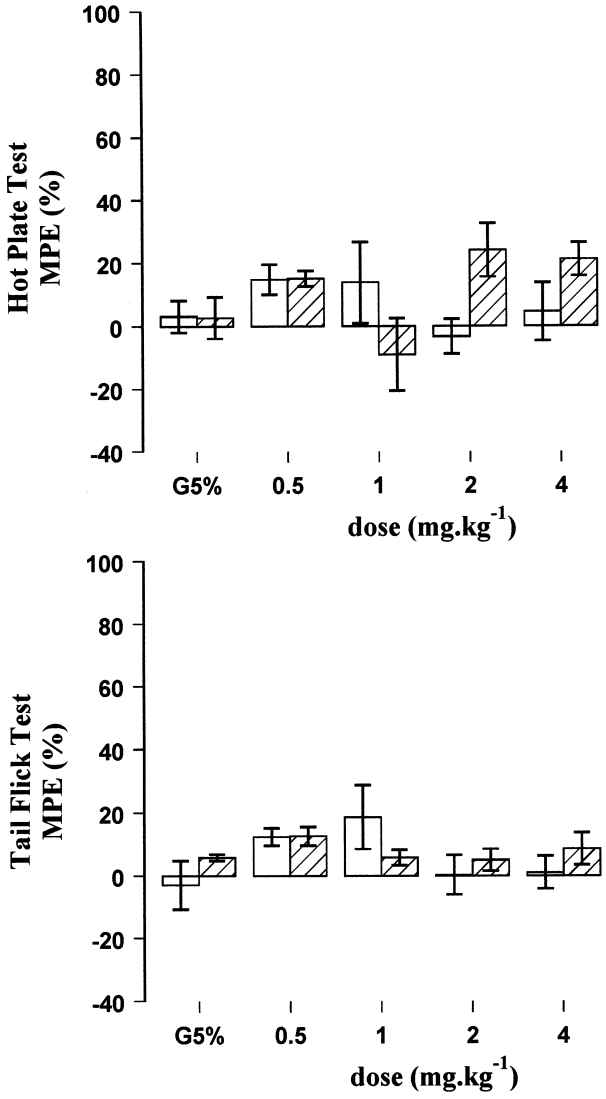

FIG. 1. Responses elicited with noxious heat expressed as maximum percentages effect (MPE; %) after injection of glucose 5% (G5%), fluoxetine (open bars), or fluvoxamine (//hatched bars). It shows MPE's in the hot-plate test (upper) and the tail-flick test (lower) after the SSRIs, which are not different from those after glucose 5%. The bars represent the mean, the error bars the SEM.

Fluoxetine and Fluvoxamine in the Thermal Algesic Tests

Analysis of the response latencies in the hot-plate and tail-flick test for effect of drug (fluoxetine or fluvoxamine) and dose, with temperature as a cofactor in a two-way ANOVA (dose and time as factors), showed no difference from glucose 5% (Fig. 1).

Effects of Fluoxetine and Fluvoxamine in the NIWR

Approximately 80 s after injection of fluoxetine or fluvoxamine, the force of withdrawal increased with a significant dose effect [ANOVA: fluoxetine, $F(5, 32) = 4.6$ $p < 0.003$; fluvoxamine, $F(5, 31) = 3.17$ $p = 0.02$] (Fig. 2). Post hoc evaluation showed that the dose-response relationships were best described using the equation of the one phase exponential association. The parameter estimates were: E_{max} 130.3 ± 6.1 and 130.4 ± 3.8%; with K 0.9 ± 0.5 and 4.5 ± 2.6 for fluoxetine and fluvoxamine, respectively. These parameter estimates did

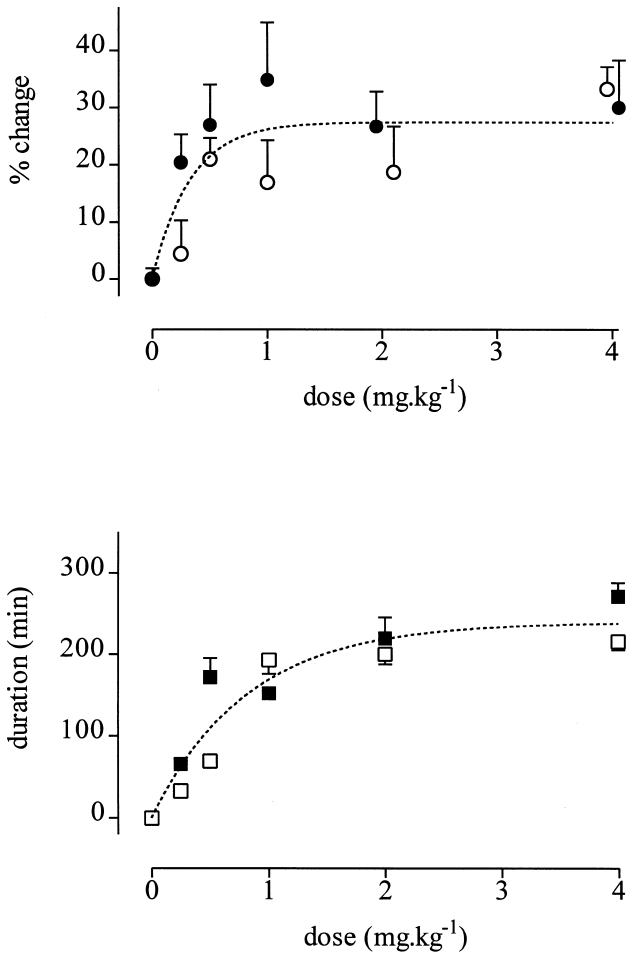

FIG. 2. Dose–effect relationship of fluoxetine (open symbols) and fluvoxamine (filled symbols) in the NIWR test (upper part). It shows the magnitude of augmented reflex responses expressed as percentage change. The lower part of this figure shows the duration of this effect in relation to the dose of fluoxetine (open symbols) and fluvoxamine (filled symbols). For both the magnitude and the duration a ceiling effect is shown. Each symbol represents the mean, the error bars the SEM.

not differ for fluoxetine and fluvoxamine, and the ceiling of increase of the withdrawal force after the two SSRIs was at approximately 130%. Thus, we found an exponential increase of effect with the dose, followed by a ceiling effect. The coefficients of variation of NIWR responses 1 h after the different doses of fluoxetine and fluvoxamine were 15.6 and 16.2%, respectively.

For the duration of the excitatory effect a significant dose effect was found [ANOVA: fluoxetine: $F(4, 28) = 66.39$, $p < 0.0001$; fluvoxamine, $F(4, 26) = 20.94$, $p < 0.0001$] (Fig. 2). Post hoc evaluation showed that again the dose–response relationships were best described using the equation of the one phase exponential association. The parameter estimates were: E_{max} 225 ± 13 and 253 ± 18%; with K 1.1 ± 0.2 and 1.3 ± 0.3 for fluoxetine and fluvoxamine, respectively. These parameter estimates did not differ for fluoxetine and fluvoxamine, and the average ceiling of duration of effect was approximately 4 h.

The injections of fluoxetine and fluvoxamine resulted in change of heart rate and diastolic IA pressure (Fig. 3), and the magnitude of these effects did not differ for the two drugs: heart rate, $F(1, 63) = 0.29$, $p = 0.6$; diastolic IA pressure, $F(1, 63)$ 5 0.00, p 5 0.9. These effects were short lasting (less than 20 s) (Fig. 4), with a significant dose effect on heart rate, $F(5, 63) = 17.42$, $p < 0.0001$, and diastolic IA pressure, $F(5, 63) = 19.05$, $p < 0.0001$ (Fig. 3). Dose–response relationships for the heart rate were best described using the equation of a first-order polynome y = ax with a = −9.8 ± 0.7; and for the diastolic blood pressure using the equation of a second order polynome $y = bx^2$ with b = −4.0 ± 0.3.

DISCUSSION

The major finding of this study is that systemic administration of the selective serotonin reuptake inhibitors fluoxetine and fluvoxamine enhanced the withdrawal responses to noxious stimulation using the NIWR method. In addition, a ceiling effect was found both for the magnitude and the duration of this excitatory effect. Otherwise, this study confirms that the two SSRIs had no effect on heat-induced reactions (29, 49). The absence of antinociceptive effect of the two SSRIs contrasts with the intrinsic analgesic effects in the same three tests after systemic administration of the nonselective reuptake inhibitor amitryptiline (20). Also, fluoxetine and fluvoxamine did not show antinociceptive effects that direct acting serotonin agonists may have (5). However, the absence of acute antinociception in the three tests does not exclude effectiveness after chronic treatment; or, that improvement of mood induced by SSRIs can result in analgesic effect in a condition of pain [cf. (26,42,47,59)].

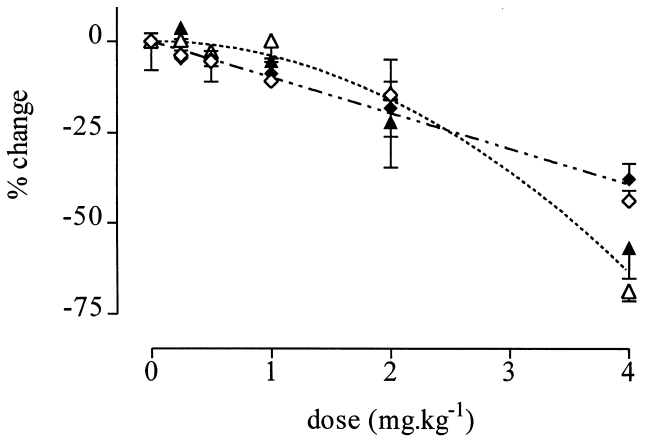

FIG. 3. The heart rate (◇; beats per minute) and diastolic intraarterial blood pressures (△; mmHg) in relation to the injections of glucose 5% and the doses of fluoxetine (open symbols) and fluvoxamine (filled symbols). The effect is expressed as the mean percentage change of the variables. Each symbol represents the mean, the error bars the SEM.

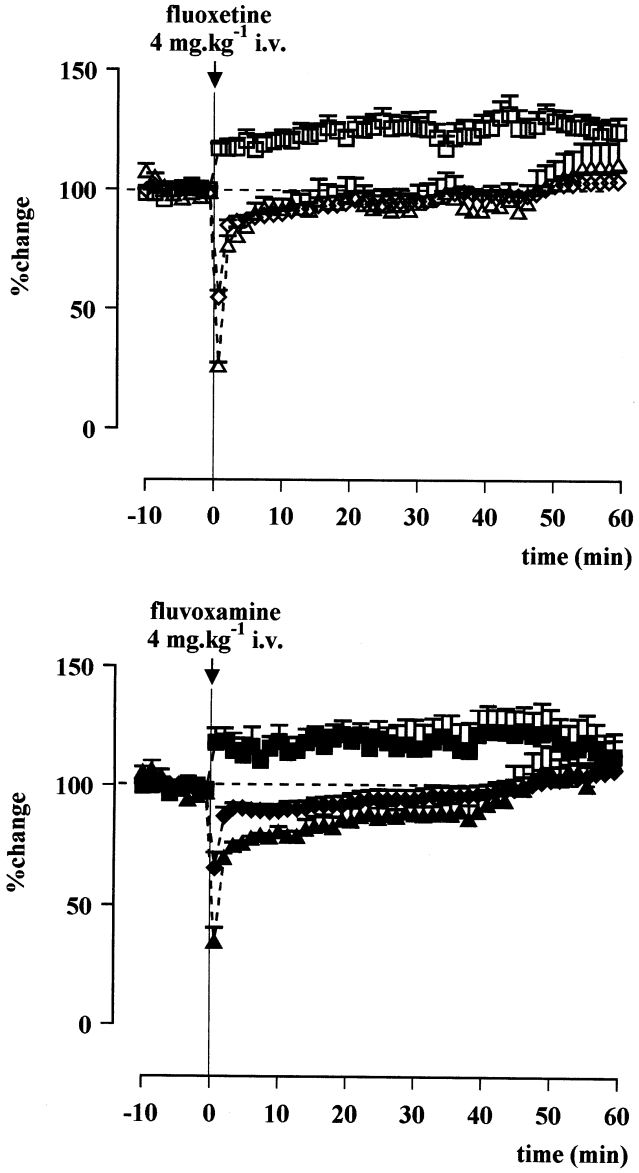

FIG. 4. Reflex responses (NIWRs; □), heart rate (bpm, ◇), and intraarterial diastolic blood pressures (mmHg; △) in relation to injections of 4 mg·kg^{-1} fluoxetine (upper part, open symbols) and 4 mg·kg^{-1} fluvoxamine (lower part, filled symbols). The effect is expressed as the mean percentage change of the variables as a function of time (minutes). It shows augmented NIWRs, and reductions of heart rates and blood pressures. The change of the latter two was short lasting, the augmentation of the NIWRs lasted several hours. Each symbol represents the mean of data, the bars the SEM.

Fluoxetine and fluvoxamine enhance the serotonergic neurotransmission by a selective block of the reuptake process. A net result of the administration of an SSRI is an acute increase in synaptic serotonin (61). This allows serotonin to act for an extended time at synaptic binding sites. The important difference between SSRIs and the direct acting agonists is that the action of SSRIs but not that of the direct acting agonists depends on neuronal release of serotonin. Thereby, SSRIs can be considered to augment basal physiological signals by amplification of serotonine's effects, but in active neuronal circuits only [e.g. (30)]. In the present study, the dose–response relationship in the NIWR responses exhibited a ceiling for magnitude and duration of effect. Maximization of reuptake inhibition after SSRI was found to relate to serotonine's stimulatory effect on autoreceptors where the elevated levels of serotonine exerted an (auto)inhibitory effect on the release of the transmitter [e.g., (21,46)]. We consider that such an effect may underlie the ceiling effect found in this study.

The present data show nociceptive responses that are dependent on the type of afferent signals (modality specific). Morphine was found to involve noradrenergic and serotonergic nociception systems variable for different types of pain [e.g., (36,37,62)]. This points to the presence of modality control in pain. The absence of effect of SSRIs in the hot-plate and tail-flick tests suggests that there is no physiological role of serotonergic transmission in thermal nociception. Rather, the SSRIs cause an enhancement of the response elicited by the electrical stimulus. This enhancement conflicts with the notion that the serotonergic descending inhibitory tracts are involved in or even cause "endogenous" analgesia (24). However, the augmentation of responses is consistent with the notion that serotonin facilitates the extraction of nociceptive information by an increase of a signal-to-noise ratio (40). According to this theory, the action of these tracts results in "contrast gain": the incoming pain signal stands out because neuronal activation by nonnoxious-related incoming information is inhibited. Functionally, this mechanism priorizes information from a painful stimulus and by the enhanced response it helps the organism to protect itself from injury (12,18,38, 39,58). Such enhancement of activity of control systems was earlier predicted to result in a high variability of responses (53), and it is noteworthy that SSRI-enhanced NIWR responses had an augmented variability as well.

The above discussion focuses on acute pain mechanisms and, in particular, those brought about by descending pathways with inhibitory serotonergic nerve endings at the spinal level. However, the action of SSRIs is not limited to the dorsal horn of the spinal cord, SSRIs are also active at other sites of the central nervous system [e.g., (30)]. Many authors reported that serotonin has excitatory effects in the somatosensory transmission (4,31,32,50,51,56). Moreover, serotonin may facilitate responses to noxious stimulation due to an enhancement of transfer of information in ascending tracts (64), or by an increase of the efficacy of transfer of information at the thalamic level (43). Finally, serotonin itself may excite motor neurons (1,57), and thereby effects not directly related to nociception may change the response to painful stimuli as well. All these mechanisms may have contributed to the augmented responses found using the NIWR method. Yet, the enhanced response we found in the NIWR method is not a self-standing finding. Clinical data indicated that fluoxetine may have antagonized the pain relief by morphine in humans (26). Therefore, one should at least take into account that SSRIs may exacerbate an acute type of pain.

Cardiovascular effects of serotonin consisted of dose-dependent reductions of heart rate and diastolic blood pressures. Central effects of serotonin were proposed to underlie the depressor cardiovascular effects (22), although a centrally administered precursor of serotonin failed to cause systematically a change of blood pressures (35). Our data do not allow to conclude whether the effects of fluoxetine and fluvoxamine are due to central effects, or caused by a direct action in blood vessels or the heart. However, the cardiovascular effect was very brief, and the enhancement of reflexes persisted for a long period. Second, the change in reflex activity was present in the absence of a relevant cardiovascular response as well. Also, cardiovascular changes of a similar magnitude by drugs that act outside the nervous system do not change the withdrawal reflex (unpublished observations). Therefore, we propose that the cardiovascular changes are not a cause for the enhancement of the withdrawal responses.

In conclusion, SSRIs may enhance natural effects of serotonin, and thereby emphasize actions that diminish or augment pain transmission. Our data show the apparent lack of an antinociceptive effect of SSRIs in heat-induced noxious stimulation. Actually, the SSRIs enhanced the withdrawal response upon noxious electrical stimulation. Thus, we may consider that serotonin is involved in the signalling of pain rather than in inhibition of pain.

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