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Sibutramine induces potential-dependent exocytotic release but not carrier-mediated release of dopamine and 5-hydroxytryptamine

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Abstract

In order to clarify the mechanism underlying the anti-obesity effects of sibutramine, we examined the effects of sibutramine on extracellular levels of dopamine and 5-hydroxytryptamine (5-HT) through microdialysis in the striatum in unanesthetized and freely moving rats. Sibutramine (5 mg/kg, oral administration (p.o.)) increased extracellular dopamine and 5-HT levels in rat striatum. The tricyclic antidepressant dosulepin (80 mg/kg, p.o. or 1 μM perfusion through the striatal probe) increased 5-HT levels only. Sibutramine-induced dopamine release was antagonized by perfusion of tetrodotoxin (1 μM) through the microdialysis probe in the striatum. However, sibutramine-induced dopamine release was not inhibited by prazosin (1 mg/kg, intraperitoneal injection (i.p.)), a suppressor of serotonergic activity in the striatum via blockade of α_1-adrenoceptors, or perfusion with nomifensine (1 μM), an inhibitor of dopamine re-uptake. These results suggest that sibutramine increases dopamine levels in the striatum by exocytotic release and not by a carrier-mediated mechanism.

Keywords: Sibutramine; Dosulepin; Microdialysis; Dopamine release; 5-HT (5-hydroxytryptamine, serotonin) release; Carrier-mediated

1. Introduction

In the initial phase of its development, sibutramine (BTS 54–524, ®Meridia) was evaluated as an antidepressant with the ability to inhibit 5-hydroxytryptamine (5-HT) and norepinephrine re-uptake. However, its body weight-reducing effect was identified in a clinical study, and it has consequently been developed as an anti-obesity drug (Bray et al., 1996; McNeely and Goa, 1998). Recently, sibutramine has been shown to be an effective and well-tolerated agent in the treatment of binge-eating disorders in obese patients (Mitchell et al., 2003).

Obesity is caused by an imbalance between caloric intake and energy expenditure (Ganong, 1999). Activation of the dopaminergic nigro-striatal pathway, which increases motor activity, is one important energy expenditure system. We have previously reported that sibutramine decreases food intake and increases spontaneous motor activity in both normal and monosodium glutamate-induced obese rats (Nakagawa et al., 2000b).

Inhibition of the re-uptake of 5-HT and norepinephrine by sibutramine might indeed be responsible for reducing food intake (Samanin and Garattini, 1993; Jackson et al., 1997). However, in hypothalamic areas, dopamine, but not 5-HT or norepinephrine, appears to play a contributory role in regulating food intake (Meguid et al., 1995; Yang and Meguid, 1995; Yang et al., 1997a,b). In addition, dopaminergic activity in rat striatum may be involved in feeding behavior (Inoue et al., 1995).

Despite the possible involvement of dopamine in food intake, Heal et al. (1992) observed that sibutramine did not exhibit dopaminomimetic activity behaviorally, in contrast to our previous findings (Nakagawa et al., 1997, 2000a, 2001). Luscombe et al. (1987) demonstrated that sibutramine or its metabolite inhibited ^{3}H-dopamine re-uptake in rat brain synaptosomes, either weakly or strongly, respectively.

However, recent studies showed that tricyclic antidepressants inhibited the re-uptake of 5-HT, which in turn activates the dopaminergic system (Santiago et al., 1998; Matsumoto et al., 1999; Engleman and Wong, 1996; Jordan et al., 1994; Tanda et al., 1994). In fact, Balcioglu and Wurtman (2000) reported that sibutramine increased not only extracellular 5-HT levels, but also dopamine levels in rat striatum and

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hypothalamus. However, the mechanism by which sibutramine stimulates dopamine release remains to be clarified experimentally, though the central dopaminergic anti-obesity effect of sibutramine is considered important.

Westerink et al. (1989) characterized three different types of drug-evoked dopamine release in conscious rats using microdialysis, an effective method for providing evidence of exocytotic or carrier-dependent monoamine release in vivo. First, action potential-dependent dopamine release was observed in animals treated with saline, haloperidol, haloperidol plus (1(2-(bis-(4-fluorophenyl)methoxy)-ethyl)-4-(3-phenylpropyl)piperazine dimaleate (GBR 12909), nomifensine, and ouabain. Second, action potential-independent release was established in the case of administration of (+)-amphetamine, glutamate, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPP^+) and 120 mmol/l Mg^{2+}. Finally, K^+-induced dopamine release was classified as tetrodotoxin-dependent and Ca^{2+}-dependent.

In the present study, to further clarify the effect of sibutramine on the dopaminergic system in the striatum, we performed brain microdialysis in rat striatum under unanesthetized and freely moving conditions, and compared effects with those on the serotonergic system.

2. Materials and methods

2.1. Animals

Male Wistar rats aged 10–12 weeks were purchased from SLC (Shizuoka, Japan). The animals were acclimatized for 1 week or more in a room at 21–23 °C and 50–70% humidity with a 12-h light/dark cycle (light on 07:00 to 19:00). The animals had free access to food and water. Handling and treatment of the animals were performed in accordance with the guidelines of the Japanese Society for Experimental Animals.

2.2. Drugs

Drugs were dissolved in distilled water for oral administration (p.o.), and in physiological saline for subcutaneous injection (s.c.) or intraperitoneal injection (i.p.) at a dose volume of 0.5 ml per 100 g body weight. Sibutramine HCl and dosulepin HCl (Prothiaden®) were synthesized by Kaken Pharmaceutical (Tokyo, Japan). Pentobarbital Na (Nembutal injection®, dissolved in distilled water) was purchased from Dainippon Pharmaceutical (Osaka, Japan). Other drugs and standards for high-performance liquid chromatography (HPLC) were obtained from RBI (Funakoshi, Tokyo, Japan) and Sigma (St. Louis, MO, USA).

2.3. Brain microdialysis

The head of a rat anesthetized with pentobarbital Na (40 mg/kg, i.p.) was fixed using a stereotaxic frame (SR-5, Narishige, Tokyo, Japan). An intracerebral dialysis guide cannula (AG-8, Eicom, Kyoto, Japan) was inserted into the striatum (A, 0.2; L, 3.0; D, 3.5) according to the rat brain atlas of Paxinos and Watson (1986) and fixed on the skull with dental cement. After operation, each animal was housed individually in a plastic cage (30 cm in diameter × 50 cm in height) for 4 days or more. On the day of the microdialysis experiment, a concentric BDP-I-8-03 dialysis probe (Eicom) was inserted into the guide cannula in the striatum, and the animal was returned to its cage. Perfusion with artificial cerebrospinal fluid (1 mM phosphate buffer, pH 7.4, containing 147 mM NaCl, 4 mM KCl and 2.3 mM $CaCl_2$) was performed at a rate of 2 μl/min under unanesthetized and freely moving conditions. Basal dialysate was collected four times at 20-min intervals. Each drug was then administered orally or intraperitoneally, and dialysate was collected every 20 min for 4 h. Four rats were used in each treatment group. On the basis of the results of previous behavioral–pharmacological and pharmaco-biochemical studies (Nakagawa et al., 1993, 1997, 2000b, 2001), doses of sibutramine and dosulepin in the present study were set at 5 mg/kg, p.o. and 80 mg/kg, p.o., respectively. A dose of 1 mg/kg, i.p. of prazosin was administered simultaneously with test drugs. Dosulepin at a concentration of 1 μM or 1 mM was continuously infused via the dialysis probe to investigate its direct effects. Perfusion of 1 μM tetrodotoxin or 1 μM nomifensine via the probe was started 60 or 120 min before the administration of test drugs, and continued until the end of experiments. Intracerebral dialysate was collected every 20-min and automatically injected into an HPLC apparatus (L-6000, Hitachi, Japan) with an auto-injector (AS-10, Eicom). Separation and quantification of both dopamine and 5-HT in dialysate were performed using a reverse-phase EICOMPAK CA 5 μm ODS 2.1ϕ × 150 mm and electrochemical detector (ECD-100, Eicom) system. Chromatography was performed with a mobile phase of 0.1 M phosphate buffer, pH 6.0, containing 400 mg/l soda 1-octanesulfonate, 50 mg/l EDTA 2Na and 20% methanol at a flow rate of 0.2 ml/min. After insertion of the probe and stabilization for 3-4 h, using the mean for three time points just before administration of drug as the baseline value (100%), the value for each time point was obtained as a percentage of the baseline value. In order to approximate a normal distribution and homoscedasticity, percentages were converted to common logarithms.

2.4. Statistical analysis

For statistical analysis of experimental data, a randomized block design and one-way layout analysis of variance were used, and comparison with the control group was performed with Dunnett's *t*-test. For examination of interactions, the single administration group and the combined administration group for each drug were tested in two-way analysis of variance (ANOVA) of repeated-measures designs (effect of the drug treatments, of time, and of their interac-

tion). Values of P less than 0.05 (two-tailed test) were considered significant.

3. Results

3.1. Effects of sibutaramine and prazosin on in vivo extracellular dopamine and 5-HT levels in rat striatum

Sibutramine (5 mg/kg, p.o.) gradually increased the levels of dopamine and 5-HT in the perfusion fluid

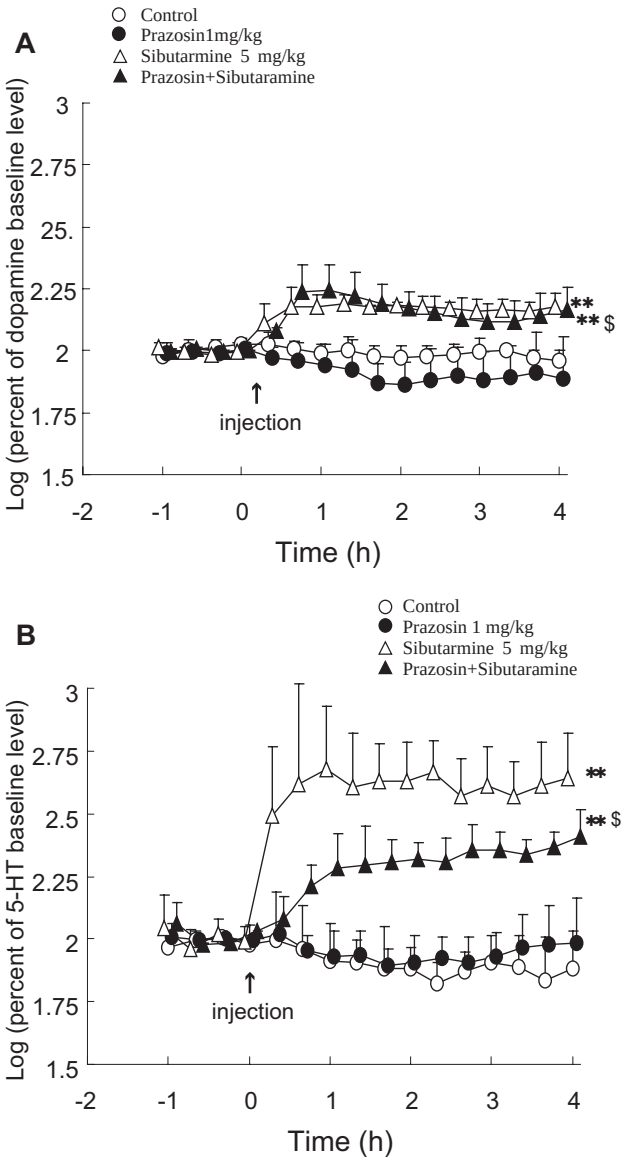

Fig. 1. Effects of prazosin (1 mg/kg, i.p., ↑) and sibutramine (5 mg/kg, p.o., ↑) on in vivo extracellular dopamine (A) and 5-HT (B) levels in rat striatum. Values are means ± S.D. (bars) of corresponding time points, expressed as log percentages (2 = 100%) of the mean pre-drug baseline values (time −40, −20 and 0 min), as measured for each group (n = 4). Y-axis indicates Log percentages. $^{**}P<0.01$ compared with control. $^{\$}P<0.05$ interaction of two-way ANOVA.

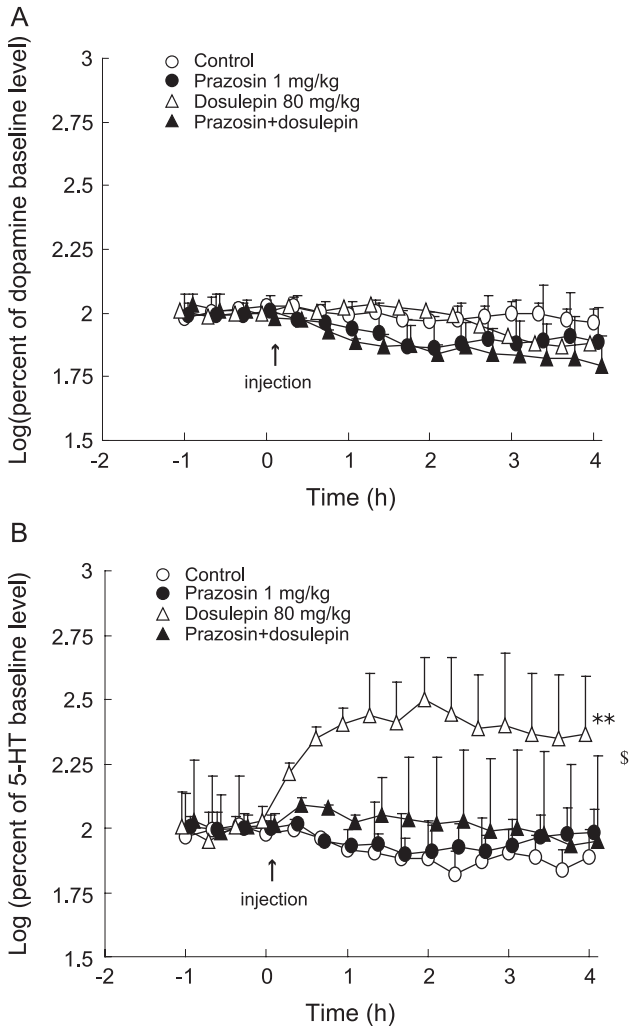

Fig. 2. Effects of prazosin (1 mg/kg, i.p., ↑) and dosulepin (80 mg/kg, p.o., ↑) on in vivo extracellular dopamine (A) and 5-HT (B) levels in rat striatum. Values are means ± S.D. (bars) of corresponding time points, expressed as log percentages (2 = 100%) of the mean pre-drug baseline values (time −40, −20 and 0 min), as measured for each group (n = 4). Y-axis indicates Log percentages. $^{**}P<0.01$ compared with control. $^{\$}P<0.05$ interaction of two-way ANOVA.

over 1–4 h to 160% and 450% of baseline values, respectively ($P<0.01$) (Fig. 1). Prazosin (1 mg/kg, i.p.) affected the levels of neither dopamine nor 5-HT in the perfusion fluid (Fig. 1). The simultaneous administration of prazosin (1 mg/kg, i.p.) and sibutramine (5 mg/kg, p.o.) caused a significant difference ($P<0.01$) in 5-HT levels, due to an interaction between prazosin and sibutramine, when analyzed by 2 × 2 factorial analysis of variance, i.e. 5-HT levels induced by simultaneous administration of sibutramine (5 mg/kg, p.o.) and prazosin were significantly lower than those obtained with sibutramine alone (5 mg/kg, p.o.). However, prazosin did not antagonize the elevation of dopamine levels caused by sibutramine (5 mg/kg, p.o.) (Fig. 1).

3.2. Effects of dosulepin and prazosin on in vivo extracellular dopamine and 5-HT levels in rat striatum

Dosulepin (80 mg/kg, p.o.) gradually increased the levels of 5-HT in the perfusion fluid over 1–4 h to 250–350% of baseline values ($P<0.01$), but did not affect the levels of dopamine (Fig. 2). 5-HT levels induced by dosulepin (80 mg/kg, p.o.) simultaneously administered with prazosin were significantly lower than those obtained with dosulepin alone (80 mg/kg, p.o.), i.e. treatment with prazosin in physiological saline significantly antagonized the elevation of 5-HT levels caused by dosulepin (80 mg/kg, p.o.) ($P<0.01$) (Fig. 2). However, an interaction between prazosin and dosulepin was not observed with respect to dopamine levels (Fig. 2).

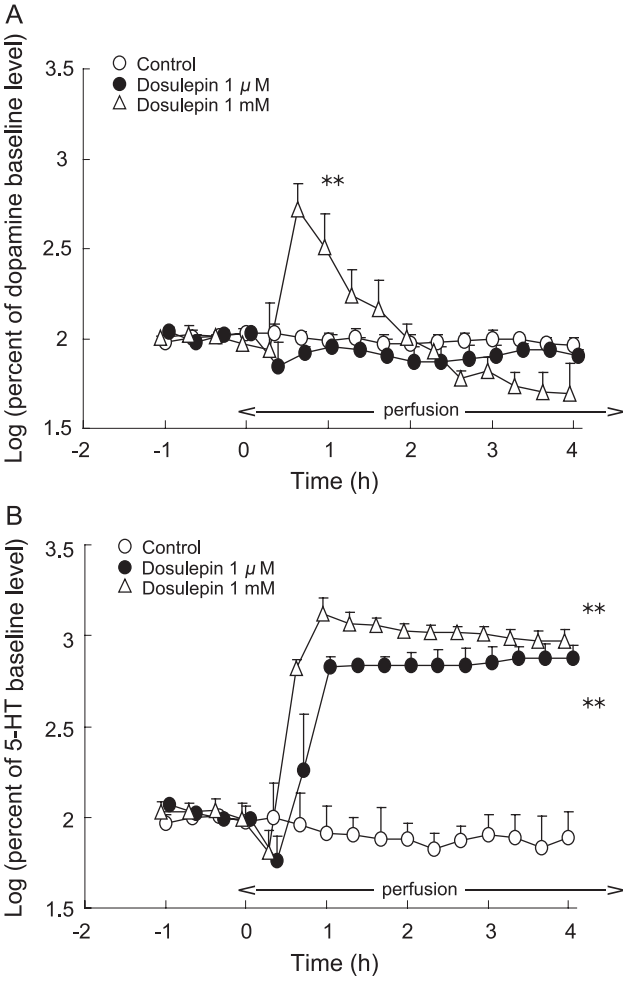

Fig. 3. Effect of continuous perfusion of dosulepin on in vivo extracellular dopamine (A) and 5-HT (B) levels in rat striatum. Values are means ± S.D. (bars) of corresponding time points, expressed as log percentages (2 = 100%) of the mean pre-drug baseline values (time −40, −20 and 0 min), as measured for each group ($n=4$). *Y*-axis indicates Log percentages. **$P<0.01$ compared with control.

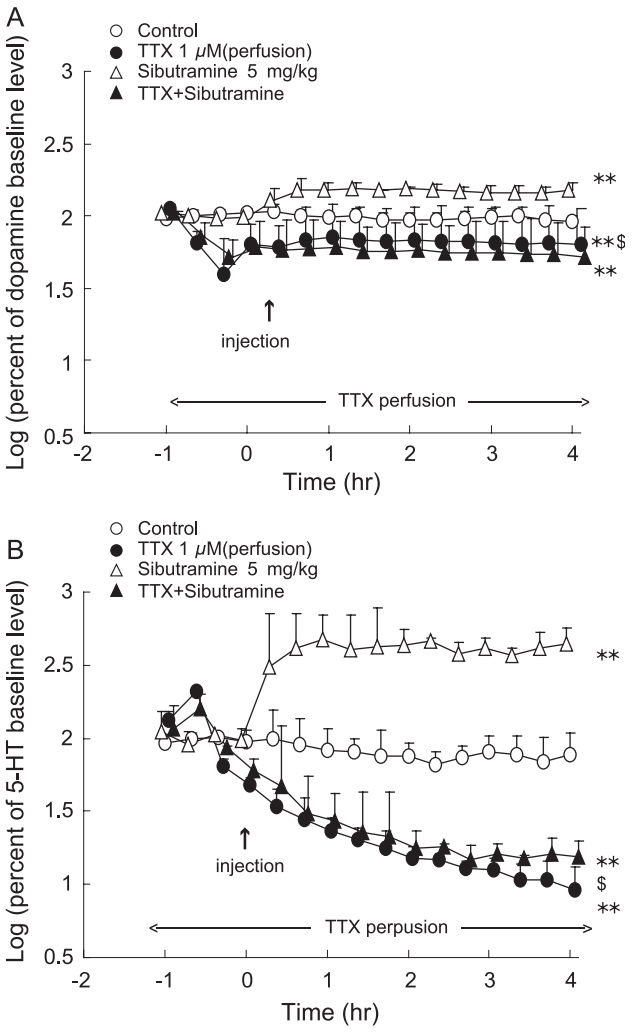

Fig. 4. Effects of tetrodotoxin perfusion (1 μM, ← →) and sibutramine (5 mg/kg, p.o., ↑) on in vivo extracellular dopamine (A) and 5-HT (B) levels in rat striatum. Data are means ± S.D. (bars) of corresponding time points, expressed as log percentages (2 = 100%) of the mean pre-drug baseline values (time −40, −20 and 0 min), as measured for each group ($n=4$). *Y*-axis indicates Log percentages. **$P<0.01$ as compared with control. $^{\$}P<0.05$ interaction of two-way ANOVA.

3.3. Effect of continuous perfusion of dosulepin on in vivo extracellular dopamine and 5-HT levels in rat striatum

Though continuous perfusion of dosulepin at a concentration of 1 μM from the striatal probe did not affect the level of dopamine, the perfusion of 1 mM transiently increased the levels of dopamine in the perfusion fluid at 1 h to 400–800% of baseline values ($P<0.01$) (Fig. 3). Continuous intrastriatal perfusion of dosulepin at concentrations of 1 μM and 1 mM gradually increased the levels of 5-HT in the perfusion fluid over 1–4 h to 650–900% and 900–2000% of baseline values, respectively ($P<0.01$) (Fig. 3).

3.4. Effect of tetrodotoxin perfusion and sibutramine on in vivo extracellular dopamine and 5-HT levels in rat striatum

Continuous perfusion of tetrodotoxin (1 μM) from the striatal probe lowered levels of dopamine and 5-HT to 30–70% and 10–70% of baseline values, respectively ($P<0.01$) (Fig. 4). Continuous perfusion of tetrodotoxin (1 μM) significantly suppressed the elevation of the levels of the two amines induced by sibutramine (5 mg/kg, p.o.) (interaction: $P<0.01$) (Fig. 4).

3.5. Effect of nomifensine perfusion and sibutramine on in vivo extracellular dopamine and 5-HT in rat striatum

Continuous perfusion of nomifensine (1 μM) from the striatal probe increased levels of dopamine and 5-HT to 25–500% and 120–200% of baseline values, respectively ($P<0.01$) (Fig. 5). Continuous perfusion of nomifensine did not significantly suppress the elevation of the levels of the two amines induced by sibutramine (5 mg/kg, p.o.) (interaction: $P<0.01$) (Fig. 5).

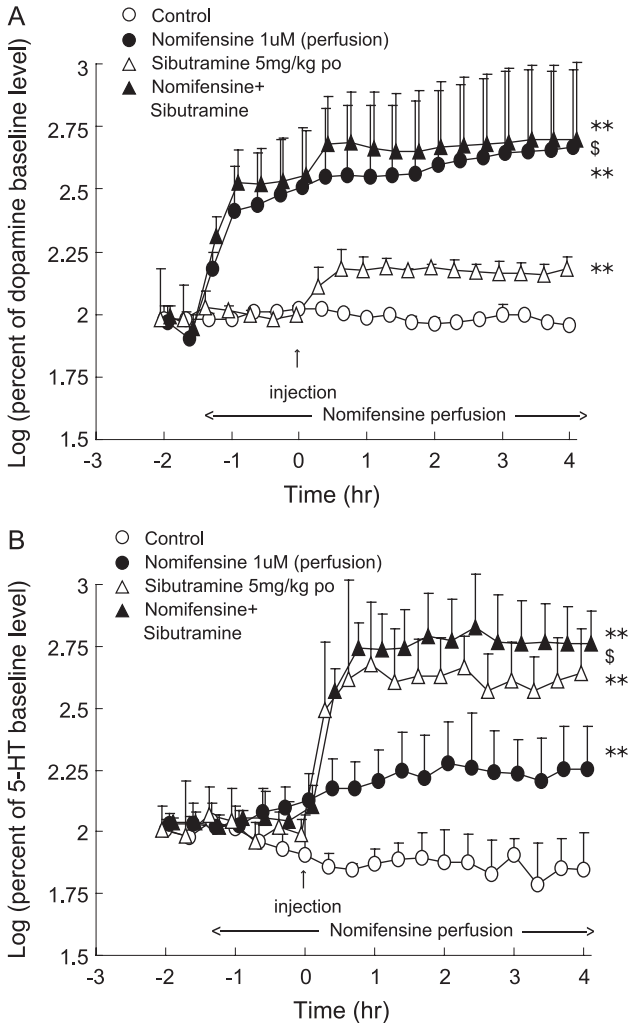

Fig. 5. Effects of nomifensine perfusion (1μM, ← →) and sibutramine (5 mg/kg, p.o., ↑) on in vivo extracellular dopamine (A) and 5-HT (B) levels in rat striatum. Values are means ± S.D. (bars) of corresponding time points, expressed as log percentages (2 = 100%) of the mean pre-drug baseline values (time −40, −20 and 0 min), as measured for each group ($n=4$). *Y*-axis indicates Log percentages. **$P<0.01$ compared with control. $^{\$}P<0.05$ interaction of two-way ANOVA.

4. Discussion

Sibutramine (5 mg/kg, p.o.) gradually increased the levels of dopamine and 5-HT in the perfusion fluid over 1–4 h to 160% and 450% of baseline values, respectively. This is consistent with the result of Balcioglu and Wurtman (2000).

In the first series of experiments, we studied the effect of prazosin on sibutramine-induced dopamine release. Prazosin blocks the somatodendritic α_1-adrenoceptors of the dorsal raphe nuclear serotonergic cells and suppresses serotonergic activity in the striatum (Rouquier et al., 1994), When given alone, prazosin affected neither 5-HT nor dopamine release, consistent with the results of other experiments performed in the nucleus accumbens (Darracq et al., 1998; Mathe et al., 1996). The increase in extracellular levels of 5-HT mediated by sibutramine was suppressed by prazosin, but the increase in dopamine levels was not suppressed. This suggests that serotonergic nerve impulse activity was involved in the release of 5-HT, but not in the release of dopamine. Thus, there are at least two possible mechanisms underlying sibutramine-induced dopamine release. One possibility is that sibutramine-induced dopamine release occurs in a carrier-dependent manner. The other possibility is that sibutramine-induced dopamine release is caused in a potential-dependent (i.e. exocytosis-dependent) manner.

In order to test the first possibility, we examined the effect of dosulepin, a tricyclic antidepressant and inhibitor of 5-HT re-uptake. Dosulepin, when systemically administered at a maximally tolerated dose (80 mg/kg, p.o.) in an in vivo study (Nakagawa et al., 1993), gradually increased the level of 5-HT over 1–4 h to 250–350% of baseline values but not the level of dopamine, and its effect on 5-HT levels was also antagonized by prazosin. Continuous intrastriatal perfusion of 1 ìM dosulepin increased the level of 5-HT without affecting dopamine levels. The perfusion of dosulepin at 1 mM, a concentration approximately 1000 times that necessary to inhibit dopamine re-uptake in vitro (Nakagawa et al., 1993), increased both 5-HT and dopamine release. These observations strongly suggest that the dopamine release induced by sibutramine is not associated with the sibutramine-induced release of 5-HT or a 5-HT receptor-mediated mechanism. In contrast to the present findings, Santiago et al. (1998) reported that the increase in dopamine levels induced by a tricyclic antidepressant, or the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), was antagonized by nomifensine, a dopamine re-uptake inhibitor. They hypothesized

that the increase in extracellular 5-HT levels and resultant activation of 5-HT_1 and 5-HT_2 receptors increased dopamine levels in the striatum. This increase was due to a carrier-mediated mechanism associated with activation of 5-HT receptors, and not due to an exocytotic mechanism. This discrepancy can be explained by the fact that serotonergic drugs such as clomipramine, dosulepin and 8-OH-DPAT cause carrier-mediated release of dopamine.

Interestingly, Jackson et al. (1997) reported that sibutramine produced hypophagia in rats, and that this hypophagic response was fully antagonized by prazosin and partially antagonized by the β_1-adrenoceptor antagonist, metoprolol, or the 5-HT receptor antagonists, metergoline (non-selective), ritanserin (5-$HT_{2A/2C}$) and *N*-(1-methyl-5-indolyl)-*N*′(3-pyridyl) urea hydrochloride (SB 200646;5-$HT_{2B/2C}$). Our results also yield the additional finding that antagonism of the sibutramine-induced decrease in food intake by prazosin may not always be a direct adrenoceptor-blocking effect. It may instead be due to suppression of serotonergic activity. It has been reported that systemic administration at low doses or local perfusion of 8-OH-DPAT into the dorsal raphe nuclei activates 5-HT_{1A} somatodendritic autoreceptors of cell of the dorsal raphe nucleus and suppresses 5-HT release in the striatum, as does prazosin (Kreiss and Lucki, 1994). A high dose of 8-OH-DPAT (1024 μg/kg, i.v.) decreased the activity of dopamine-secreting cells in the substantia nigra (Arborelius et al., 1993). Such a change could be prevented by systemic pretreatment with raclopride, a selective dopamine D_2 receptor antagonist, yielding impulse-mediated (potential-dependent Na^+ channel-mediated) dopamine release (exocytotic release) (Arborelius et al., 1993). In addition, systemic administration of 50 μg/kg, s.c., but not of 1000 μg/kg, s.c., 8-OH-DPAT attenuated the increase in extracellular dopamine induced by 1.0 mg/kg, s.c., amphetamine (Ichikawa et al., 1995) Concerning such a biphasic dose–response curve for 8-OH-DPAT in vivo, it is possible that at high doses, 8-OH-DPAT may lose selectivity for 5-HT_{1A} receptor-mediated regulation of dopamine synthesis. Alternatively, this agent may modulate other systems, e.g. inhibit monoamine re-uptake and/or stimulate carrier-mediated release of dopamine (Johnson et al., 1993; Ichikawa et al., 1995; Santiago et al., 1998; Assie and Koek, 1996).

Based on findings from a previous report (Melega et al., 1995), methamphetamine can be regarded as a carrier-mediated dopamine releaser, which increases striatal extracellular dopamine and 5-HT levels. These authors showed, in in vitro studies with radiolabeled amphetamine in nanomole concentrations, that accumulation patterns in rat brain synaptosomes (Zaczek et al., 1991) correlated with dopaminergic terminal density (e.g. striatum>cortex). Selective accumulation was attributed to methamphetamine uptake into dopaminergic terminals via a high-affinity, saturable, dopamine uptake mechanism. These data implied that the mechanism of action of methamphetamine at the presynaptic terminal may be related to a direct interaction with the uptake carrier, leading subsequently to drug sequestration within these structures (Zaczek et al., 1990). Under these circumstances, it might be argued that carrier-mediated sequestration would be delayed, relative to overall striatal accumulation. At present, this issue remains unresolved, but the apparent delay in the observed dopamine response indicates a drug–brain interaction that is time dependent and yet independent of the overall drug concentration in the striatum.

8-OH-DPAT did not suppress the increase in extracellular levels of dopamine induced by methamphetamine (Ichikawa et al., 1995). However, pretreatment with 8-OH-DPAT suppressed the increase in extracellular levels of both amines (dopamine and 5-HT) caused by sibutramine (unpublished data). Gundlah et al. (1997) obtained similar results concerning 5-HT levels in the hypothalamus. These results suggest that a carrier-mediated mechanism is probably not involved in sibutramine-induced dopamine release. Furthermore, continuous intrastriatal perfusion of a dopamine re-uptake inhibitor, nomifensine (1 μM), failed to suppress sibutramine-induced increases in dopamine levels, although increases in carrier-mediated dopamine release have been shown to be completely blocked by nomifensine (Westerink et al., 1987; Butcher et al., 1988). These observations strongly suggest that carrier-mediated release is not involved in the sibutramine-induced dopamine release.

Finally, to test the second possibility, i.e. that the sibutramine-induced dopamine release is caused in a potential-dependent (i.e. exocytosis-dependent) manner, we examined the effects of tetrodotoxin on sibutramine-induced dopamine release. Local administration of tetrodotoxin, which suppresses action potentials by blocking voltage-dependent Na^+ channels, does not suppress the elevation of extracellular levels of monoamines induced by methamphetamine or D-amphetamine, which act in a carrier-dependent fashion. Tetrodotoxin suppresses the increase in extracellular monoamine levels induced by monoamine re-uptake inhibitors such as amitriptyline, imipramine, cocaine, nomifensine and bupropion. Tetrodotoxin is therefore frequently used for examining the mechanisms of action of monoaminergic drugs. The increase in extracellular levels of dopamine and 5-HT induced by sibutramine in this study was completely suppressed by continuous perfusion of tetrodotoxin.

In conclusion, our results strongly suggest that sibutramine induces potential-dependent exocytotic release, but not carrier-mediated release, of dopamine and 5-HT.

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