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Modulation of Dialysate Levels of Dopamine, Noradrenaline, and Serotonin (5-HT) in the Frontal Cortex of Freely-Moving Rats by (-)-Pindolol Alone and in Association with 5-HT Reuptake Inhibitors: Comparative Roles of β-Adrenergic, 5-HT_{1A}, and 5-HT_{1B} Receptors

Alain Gobert, Ph.D. and Mark J. Millan, Ph.D.

(-)-Pindolol, which possesses significant affinity for 5-HT_{1A}, 5-HT_{1B}, and $\beta_{1/2}$-adrenergic receptors (AR)s, dose-dependently increased extracellular levels of dopamine (DA) and noradrenaline (NAD) versus 5-HT, in dialysates of the frontal cortex (FCX), but not accumbens and striatum, of freely-moving rats. In distinction, the preferential β_1-AR antagonist, betaxolol, and the preferential β_2-AR antagonist, ICI118,551, did not increase basal levels of DA, NAD, or 5-HT. Further, they both dose-dependently and markedly blunted the influence of (-)-pindolol upon DA and NAD levels. The selective 5-HT_{1A} receptor antagonist, WAY100,635, slightly attenuated the (-)-pindolol-induced increase in DA and NAD levels, while the selective 5-HT_{1B} antagonist, SB224,289, was ineffective. These data suggest that (-)-pindolol facilitates frontocortical dopaminergic (and adrenergic) transmission primarily by activation of $\beta_{1/2}$-ARs and, to a lesser degree, by stimulation of 5-HT_{1A} receptors, whereas 5-HT_{1B} receptors are not involved. (-)-Pindolol potentiated the increase in FCX levels of 5-HT elicited by the 5-HT reuptake inhibitors, fluoxetine and duloxetine, and also enhanced their ability to elevate FCX levels of DA—though not of NAD. In contrast to (-)-pindolol, betaxolol and ICI118,551 did not affect the actions of fluoxetine, whereas both WAY100,635 and SB224,289 potentiated the increase in levels of 5-HT—but not *DA or NAD levels—elicited by fluoxetine. In conclusion, (-)-pindolol modulates, both alone and together with 5-HT reuptake inhibitors, dopaminergic, adrenergic, and serotonergic transmission in the FCX via a complex pattern of actions at $\beta_{1/2}$-ARs, 5-HT_{1A}, and 5-HT_{1B} receptors. These findings have important implications for clinical studies of the influence of (-)-pindolol upon the actions of antidepressant agents.*

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Although selective 5-HT reuptake inhibitors (SSRI)s present significant advantages to tricyclic antidepressant (AD)s in terms of their improved tolerance, they share the delayed onset of action and limited efficacy of first-generation ADs (Frazer 1997). Correspondingly,

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there is considerable interest in strategies which may allow for a more rapid onset of therapeutic benefit (Blier and de Montigny 1994; Frazer 1997; Lucki et al. 1994; Nemeroff 1997). In this regard, it has been hypothesized that a desensitization of 5-HT_{1A} autoreceptors (localized on the dendrites of serotonergic neurones) underlies the lack of an immediate response to AD administration. According to this hypothesis (Artigas et al. 1996; Blier et al. 1990), the delay to onset of action of SSRIs (and other drugs inhibiting 5-HT uptake) may be explained by their propensity to increase extracellular levels of 5-HT not only postsynaptically, but also presynaptically at inhibitory 5-HT_{1A} autoreceptors localized on the dendrites of serotonergic cell bodies in raphe nuclei (Davidson and Stamford 1995; Rutter et al. 1995). This simultaneous activation of 5-HT_{1A} autoreceptors diminishes the activity of serotonergic neurones, thereby braking the postsynaptic increase in 5-HT levels.

The progressive desensitization of 5-HT_{1A} autoreceptors is accompanied by a gradual reinforcement in serotonergic transmission and the development of therapeutic AD actions (Artigas et al. 1996). Correspondingly, the acute blockade of 5-HT_{1A} autoreceptors should mimic processes of desensitization. Indeed, selective 5-HT_{1A} receptor antagonists, such as WAY100,635, as well as the 5-HT_{1A} receptor ligand, (-)-pindolol, potentiate the influence of SSRIs upon postsynaptic levels of 5-HT in corticolimbic structures in rats (Arborelius et al. 1996; Artigas et al. 1996; Galloway 1996; Gartside et al. 1995; Gobert et al. 1997b; Hjorth 1996; Hjorth et al. 1996). Furthermore, blockade of 5-HT_{1A} autoreceptors potentiates certain behavioural actions of SSRIs (Detke et al. 1996; Jackson et al. 1997; Millan et al. 1998a; Mitchell and Redfern 1997; Trillat et al. 1998). This intriguing hypothesis provides, thus, a rational, theoretical basis for clinical studies of the co-administration of 5-HT_{1A} autoreceptor antagonists with AD agents. Indeed, as concerns therapeutic AD actions, co-treatment with (-)-pindolol and SSRIs has been reported as beneficial in several—though not all—clinical studies (Berman et al. 1997; DeBattista et al. 1998; McAskill et al. 1998; Nemeroff 1997).

There exist, however, conflicting experimental data concerning the desensitization of 5-HT_{1A} autoreceptors upon their chronic stimulation by treatment with SSRIs or direct agonists (Artigas et al. 1996; Hjorth and Auerbach 1994; Kreiss and Lucki 1997; Le Poul et al. 1995). Furthermore, several questions remain concerning the mechanism(s) underlying the apparent ability of (-)-pindolol to enhance the actions of AD agents. *First*, possibly depending upon the level of extracellular 5-HT, (-)-pindolol has been shown to exert mixed agonist and antagonist actions at 5-HT_{1A} autoreceptors (Clifford et al. 1998; Corradetti et al. 1998; Romero et al. 1996; Lejeune and Millan unpublished observations) (see Discussion). *Second*, serotonergic neurones also bear inhibitory 5-HT_{1B} receptors on their terminals (and, possibly, cell bodies) which may also adapt upon chronic treatment with SSRIs, and (-)-pindolol is also an antagonist at these sites in the rat (Adham et al. 1992; Assie and Koek 1996; Bourin et al. 1998; Hoyer and Schoeffter 1991; Millan et al. in press). *Third*, (-)-pindolol is a potent partial agonist at β-ARs (Frishman 1983; Hoffman and Lefkowitz 1996), a role of which has been implicated in depressive states (O'Donnell et al. 1994; Thiessen et al. 1990; Zohar et al. 1987). *Fourth*, 5-HT_{1A}, 5-HT_{1B} and β-ARs may all play modulatory roles in controlling the activity of dopaminergic and adrenergic projections to the FCX (Gobert et al. 1998; Kelland and Chiodo 1996; Lejeune et al. 1998; Millan et al. 1997; Misu and Kubo 1986; Murugaiah and O'Donnell 1995), and a perturbation in the activity of these pathways is implicated in the emotional and cognitive deficits of depressive states (Caldecott-Hazard et al. 1991; Goodwin 1997; Leonard 1997; Zacharko and Anisman 1991).

These observations raise the question of a potential influence of (-)-pindolol upon depressive states via an interaction with dopaminergic and/or adrenergic mechanisms in the FCX. Thus, herein, we characterized the potential influence of (-)-pindolol, both alone and together with SSRIs, upon frontocortical dopaminergic, adrenergic, and serotonergic transmission. Inasmuch as (-)-pindolol possesses significant affinity for $\beta_{1/2}$-AR, 5-HT_{1A}, and 5-HT_{1B} receptors (Table 1) (*vide-supra*), we evaluated their respective roles by examining whether selective antagonists at these sites either mimicked or blocked the effects of (-)-pindolol. A preliminary account of some of these findings has appeared in Abstract form (Lejeune et al. 1998).

METHODS

Male Wistars rats (Iffa Credo, l'Arbresle, France) of 200–220 g were allowed free access to food and water

Table 1. Binding Affinities of (-)-Pindolol at Cloned, Human (h), Native Rat (r), and Native guinea pig (gp) receptor subtypes

	h5-HT_{1A}	**r5-HT_{1B}**	**gp5-HT_{1B}**	**h5-HT_{1B}**	**h5-HT_{1D}**	**rβ_1**	**rβ_2**
(-)-Pindolol	8.2[a]	7.2[b]	5.9[c]	<5.0[c]	5.2[c]	9.2[b]	9.5[b]

Values are pKis. Data are from Newman-Tancredi et al. (1998)[a], Tsuchihashi et al. (1990)[b], and Newman-Tancredi and Millan unpublished observations[c].

and housed singly. Laboratory temperature was 21 ± 1°C and humidity 60 ± 5%. There was a 12h/12h light/dark cycle (lights on at 7:30 a.m.). All animal use procedures conformed to international european ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

Rats were implanted under pentobarbital anaesthesia (60 mg/kg, i.p.) with a guide cannula in the FCX or in both the nucleus accumbens and the contralateral striatum as described (Gobert et al. 1998; Millan et al. 1997). Five days later, a concentric dialysis probe—4 mm length (FCX and striatum) and 2 mm length (accumbens), 0.24 mm, o.d.—was lowered into position and perfused at 1 μl/min with a phosphate-buffered solution of NaCl, 147.2 mM; KCl, 4 mM; and $CaCl_2$, 2.3 mM (pH 7.3). Two hours after implantation, samples were taken every 20 min and three basal samples were collected before one or two subcutaneous injections separated by 20 min. Serotonin, DA and NAD were simultaneously quantified by HPLC/coulometric detection (ESA 5014, Coulochem II; $E_1 = -90$ mV and $E_2 = +280$ mV). Monoamines were separated using a Hypersil column (C18; 150 × 4.6 mm; particle size 5 μm) maintained at 34°C and a mobile phase comprising 75 mM NaH_2PO_4, 20 μM EDTA, 1 mM sodium decanesulfonate, 17.5% methanol, and 0.01% triethylamine (pH 5.70; flow rate 2 ml/min). The sensitivity of the assay for 5-HT, DA and NAD was between 0.1 and 0.2 pg per sample, respectively. All data are expressed as means ±S.E.M.s. Monoamine levels were expressed as a function of mean basal pre-injection values (= 100 %). ANOVA with sampling time as the repeated within-subject factor was performed.

Chemicals and Drugs

All drugs were injected subcutaneously (s.c.) in a volume of 1.0 ml/kg. Drugs were dissolved in sterile water, plus a few drops of lactic acid, if necessary. Drug sources were as follow: (-)-pindolol, base, and 8-OH-DPAT, HBr (Research Biochemical International, Natick, MA); (±)-pindolol, base, 1-decanesulfonate, NaCl (Sigma Chimie, St. Quentin Fallavier, France); betaxolol, HCl (Synthélabo, Bagneux, France); ICI118,551 {erythro-*d*, *l*-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol}, HCl (Zeneca, Macclesfield, UK); duloxetine, HCl (Lilly, Indianapolis, USA); GR46611 {(3-[3-(2-dimethylaminoethyl)-1H-indol-5-yl]-N-(4-methoxybenzyl) acrylamid)}, base (Tocris Cookson, Bristol, U.K.); fluoxetine HCl, SB224,289 {5,1′-methyl-5-((2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-yl) carbonyl)-2,3,6,7-tetrahydrospiro (furo(2,3-f) indol-3,4′-piperidine)}, base, and WAY100,635 {(N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclo-hexane-carboxamide}, 3HCl were synthesized by Servier chemists (G. Lavielle and J.-L. Peglion).

RESULTS

Influence of (-)-Pindolol upon Resting Levels of 5-HT, DA, and NAD in FCX as Compared to Striatum and Nucleus Accumbens

Administration of vehicle did not influence extracellular levels of DA or 5-HT but elicited a mild and transient increase in dialysate levels of NAD (see Gobert et al. 1998; Millan et al. 1997) (Figure 1). As shown in Figure 1, (-)-pindolol elicited a sustained and marked increase in levels of both DA and NAD. This action was expressed dose-dependently over a dose-range of 2.5–20.0 mg/kg, s.c. In contrast to DA and NAD, (-)-pindolol did *not* elevate levels of 5-HT. Indeed, as shown in the Figure 2, in certain experiments, there was a very mild, but significant, decrease in levels of 5-HT. In analogy to (-)-pindolol, racemic (±)-pindolol (10.0 mg/kg, s.c.) similarly increased FCX levels of DA and NAD (Figure 1). In contrast to FCX, (-)-pindolol (20.0) exerted little influence upon dialysate levels of DA in the nucleus accumbens and striatum (Figure 1).

Influence of (-)-Pindolol upon the Actions of the 5-HT_{1A} Agonist, 8-OH-DPAT, and of the 5-HT_{1B} Agonist, GR46611

The selective 5-HT_{1A} agonist, 8-OH-DPAT (0.16), markedly suppressed dialysate levels of 5-HT, an action which was prevented by (-)-pindolol (10.0) (Figure 2). The agonist 8-OH-DPAT also provoked a marked increase in FCX levels of DA and, in the presence of (-)-pindolol, this action was slightly attenuated. (Indeed, there was a tendency for levels of DA to be even lower in the (-)-pindolol/8-OH-DPAT group as compared to the vehicle/8-OH-DPAT group, although this difference was not statistically significant. Possibly, this reflects an interaction between 5-HT_{1A} and β-AR receptor-mediated mechanisms controlling DA release in FCX). In addition, 8-OH-DPAT elicited a pronounced increase in NAD levels, which was *not* significantly modified by (-)-pindolol (Figure 2). The preferential 5-HT_{1B} agonist, GR46611 (10.0), diminished dialysate levels of 5-HT. This action was blocked by (-)-pindolol (Figure 2). Further, GR46611 did not modify levels of DA or NAD. The influence of GR46611 and (-)-pindolol in combination upon DA and NAD levels was equivalent to that of (-)-pindolol alone (Figure 2). The selective 5-HT_{1A} antagonist, WAY100,635 (0.16), abolished the influence of 8-OH-DPAT (0.16) upon FCX levels of 5-HT without affecting the influence of GR46611 (10.0) (Gobert et al. 1998 and not shown). In an opposite fashion, the selective 5-HT_{1B} antagonist, SB224,289 (2.5), abolished the

action of GR46611 (10.0) without affecting that of 8-OH-DPAT (0.16) (data not shown).

Influence of Receptor-Selective Agents upon the Actions of (-)-Pindolol

In the presence of the preferential β_1-AR antagonist, betaxolol, and the preferential β_2-AR antagonist, ICI118,551, the influence of (-)-pindolol (10.0) upon levels of DA was dose-dependently and markedly blunted in each case (Figure 3). They also significantly, though to a lesser degree, abrogated the influence of (-)-pindolol upon NAD levels (Figure 3). These actions were maintained throughout the dialysis period. The selective 5-HT_{1A} receptor antagonist, WAY100,635 (0.16), was inactive alone yet slightly and significantly diminished the increase in dialysate levels of DA and NAD provoked by (-)-pindolol (10.0) (Figure 4). In contrast, the selective 5-HT_{1B} receptor antagonist, SB224,289 (2.5), failed to modify the action of (-)-pindolol (10.0), and likewise did not affect basal levels of DA or NAD (Figure 4).

Influence of (-)-Pindolol upon the Actions of Fluoxetine and Duloxetine

The doses of fluoxetine and duloxetine selected for these studies, 5.0 and 10.0 mg/kg, s.c., respectively, were based upon their ability to elicit increases in dialysate levels of 5-HT in FCX of a comparable magnitude (Figure 5), thereby facilitating comparisons of the modulation of their actions by (-)-pindolol. In addition, fluoxetine and duloxetine each elicited a pronounced elevation in levels of DA (Figure 5). Fluoxetine evoked an increase in NAD levels of a similar magnitude to 5-HT, whereas the influence of duloxetine upon NAD levels was more accentuated—in line with its high affinity for NAD reuptake sites (Engleman et al. 1995) (Figure 5). Pretreatment with (-)-pindolol (10.0) enhanced the in-

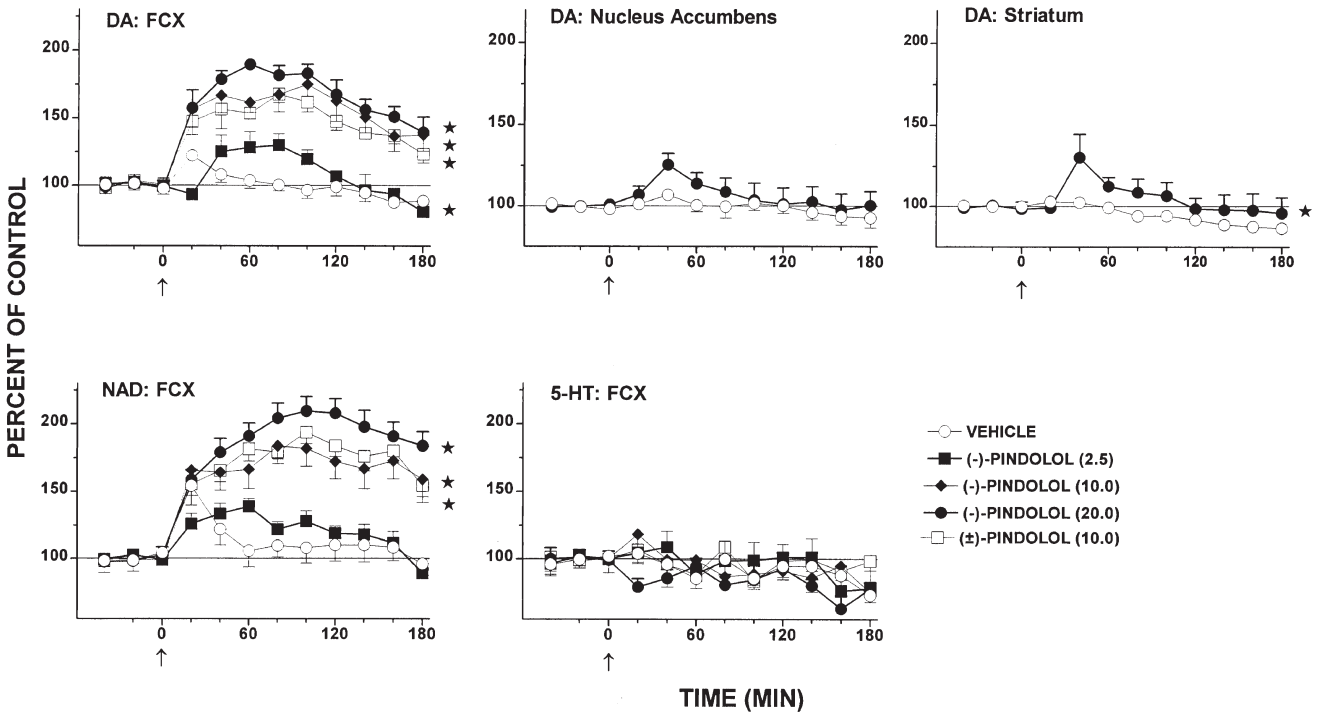

Figure 1. Influence of (-)-pindolol upon dialysate levels of DA, NAD and 5-HT in the FCX as compared to striatum and nucleus accumbens of freely-moving rats. Data are means ± S.E.M.s of 5-HT, DA, and NAD levels expressed as a percentage of basal pre-injection values (= 100%). N = 5–11 per value. Levels were 0.74 ± 0.07, 1.21 ± 0.08, and 1.28 ± 0.12 pg/20 μl dialysate for 5-HT, DA, and NAD, respectively, in FCX and 12.9 ± 1.6 and 8.4 ± 1.3 pg/20 μl dialysate for DA in accumbens and striatum, respectively. For comparison of individual values with vehicle-treated group, ANOVA (40–180 min) as follows: *DA, FCX*: (-)-pindolol (2.5), $F(1,17) = 4.5$, $p < .05$; (-)-pindolol (10.0), $F(1,22) = 39.1$, $p < .01$; (-)-pindolol (20.0), $F(1,17) = 111.4$, $p < .01$; and (±)-pindolol (10.0), $F(1,16) = 62.7$, $p < .01$. *DA, accumbens*: $F(1,17) = 0.9$, $p > .05$. *DA, striatum*: $F(1,16) = 5.8$, $p < .05$. *NAD, FCX*: (-)-pindolol (2.5), $F(1,15) = 1.1$, $p > .05$; (-)-pindolol (10.0), $F(1,19) = 19.5$, $p < .01$; (-)-pindolol (20.0), $F(1,15) = 49.2$, $p < .01$; and (±)-pindolol (10.0), $F(1,15) = 36.1$, $p < .01$. *5-HT, FCX*: (-)-pindolol (2.5), $F(1,10) = 0.2$, $p > .05$; (-)-pindolol (10.0), $F(1,11) = 0.1$, $p > .05$; (-)-pindolol (20.0), $F(1,10) = 1.7$, $p > .05$; and (±)-pindolol (10.0), $F(1,10) = 1.2$, $p > .05$. *$p < .05$ for drug versus vehicle.

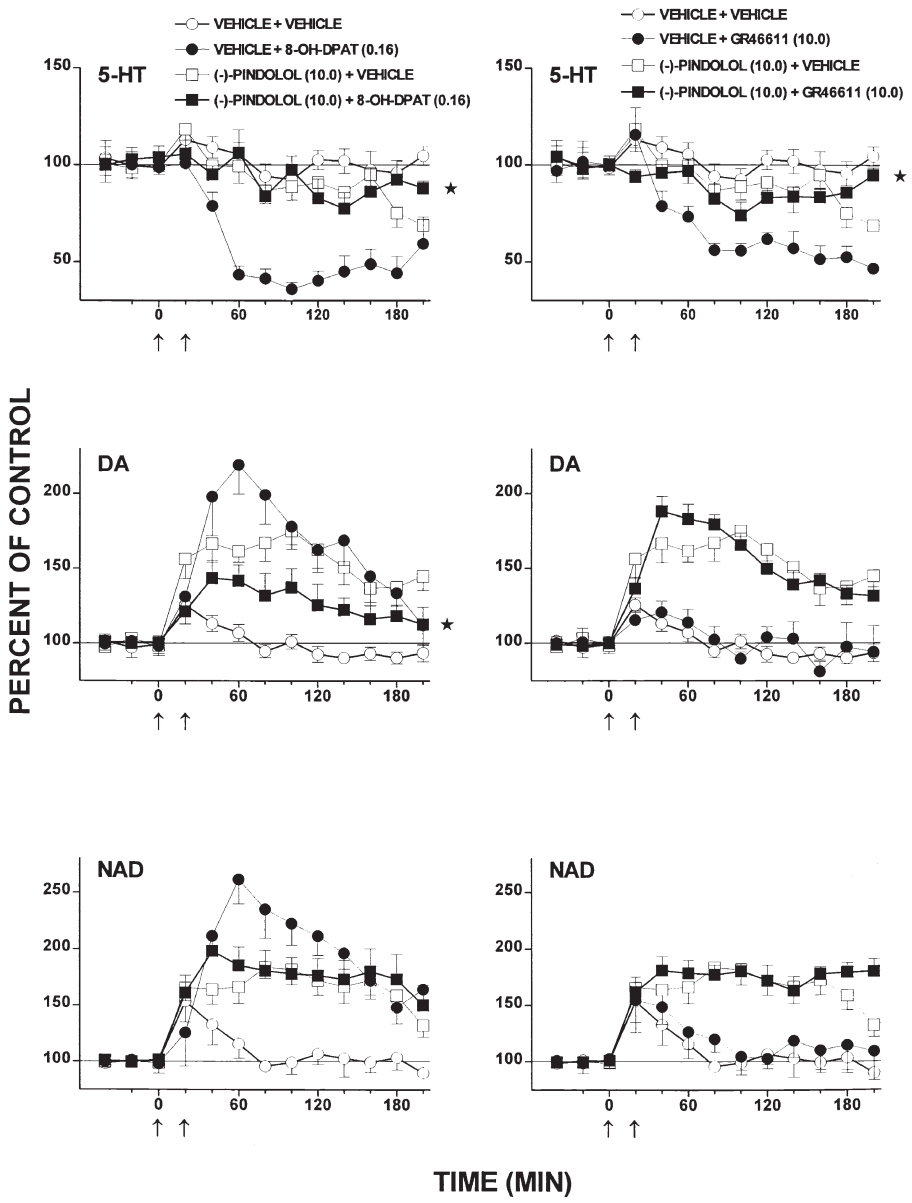

Figure 2. Influence of (-)-pindolol upon modulation of FCX levels of DA and NAD as compared to 5-HT by 8-OH-DPAT and GR46611. Data are means ± S.E.M.s of 5-HT, DA, and NAD levels expressed as a percentage of basal pre-injection values (= 100%). N = 5–11 per value. ANOVAs were performed over 40–200 min. *5-HT*: (-)-pindolol + vehicle vs. vehicle + vehicle, $F(1,16) = 5.0$, $p < .05$; vehicle + 8-OH-DPAT vs. vehicle + vehicle, $F(1,20) = 59.5$, $p < .01$; (-)-pindolol + 8-OH-DPAT vs. vehicle + 8-OH-DPAT, $F(1,15) = 25.9$, $p < .01$; vehicle + GR46611 vs. vehicle + vehicle, $F(1,14) = 41.0$, $p < .01$; and (-)-pindolol + GR46611 vs. vehicle + GR46611, $F(1,9) = 53.8$, $p < .01$. *DA*: (-)-pindolol + vehicle vs. vehicle + vehicle, $F(1,19) = 35.6$, $p < .01$; vehicle + 8-OH-DPAT vs. vehicle + vehicle, $F(1,19) = 25.1$, $p < .01$; (-)-pindolol + 8-OH-DPAT vs. vehicle + 8-OH-DPAT, $F(1,15) = 4.3$, $p < .05$; vehicle + GR46611 vs. vehicle + vehicle, $F(1,13) = 0.4$, $p > .05$; and (-)-pindolol + GR46611 vs. (-)-pindolol + vehicle, $F(1,16) = 0.1$, $p > .05$. *NAD*: (-)-pindolol + vehicle vs. vehicle + vehicle, $F(1,17) = 18.3$, $p < .01$; vehicle + 8-OH-DPAT vs. vehicle + vehicle, $F(1,17) = 33.9$, $p < .01$; (-)-pindolol + 8-OH-DPAT vs. vehicle + 8-OH-DPAT, $F(1,14) = 1.3$, $p > .05$; vehicle + GR46611 vs. vehicle + vehicle, $F(1,12) = 0.8$, $p > .05$; and (-)-pindolol + GR46611 vs. (-)-pindolol + vehicle, $F(1,15) = 0.4$, $p > .05$. Asterisks indicate significance of differences of (-)-pindolol + 8-OH-DPAT and (-)-pindolol + GR46611 to vehicle + 8-OH-DPAT and to vehicle + GR46611 values, respectively. * $p < .05$.

fluence of fluoxetine and duloxetine upon dialysate levels of 5-HT by ca. 2-fold in each case (Figure 5). The influence of fluoxetine and duloxetine upon DA levels was also more pronounced in the presence of (-)-pindolol, with the total effect corresponding to an (apparently) additive influence of (-)-pindolol plus fluoxetine and (-)-pindolol plus duloxetine, respectively (Figure 5). In contrast, the influence of fluoxetine and duloxe-

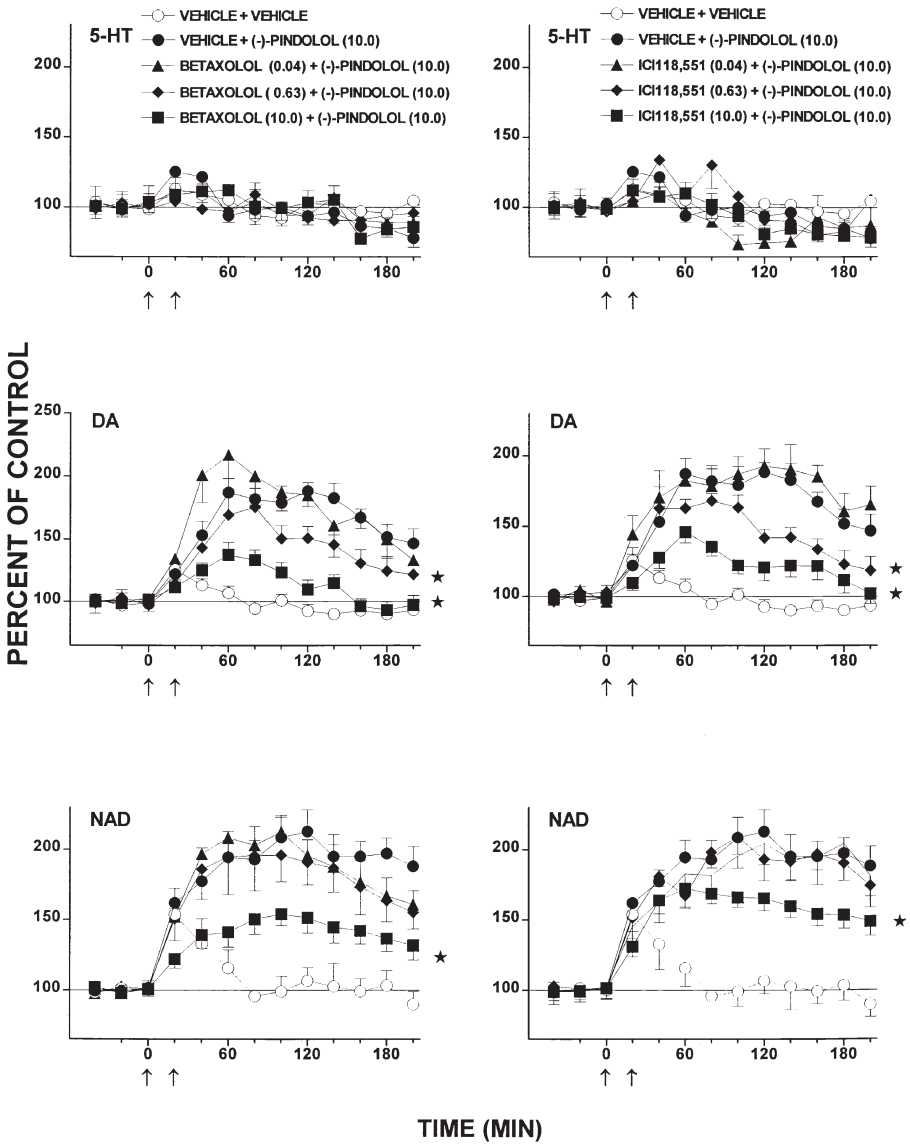

Figure 3. Influence of the β_1- and β_2- AR antagonists, betaxolol and ICI118,551, respectively, upon modulation of FCX levels of DA and NAD as compared to 5-HT by (-)-pindolol. Data are means ± S.E.M.s of 5-HT, DA, and NAD levels expressed as a percentage of basal pre-injection values (= 100%). N = 5–11 per value. ANOVAs were performed over 40–200 min. *5-HT*: vehicle + (-)-pindolol vs. vehicle + vehicle, $F(1,19) = 1.0$, $p > .05$; betaxolol (10.0) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,16) = 0.3$, $p > .05$; betaxolol (0.63) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,14) = 0.1$, $p > .05$; betaxolol (0.04) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,13) = 0.2$, $p > .05$; ICI118,551 (10.0) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,16) = 0.5$, $p > .05$; ICI118,551 (0.63) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,13) = 2.5$, $p > .05$; and ICI118,551 (0.04) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,12) = 1.5$, $p > .05$. *DA*: vehicle + (-)-pindolol vs. vehicle + vehicle, $F(1,18) = 85.2$, $p < .01$; betaxolol (10.0) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,16) = 34.2$, $p < .01$; betaxolol (0.63) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,14) = 5.4$, $p < .05$; betaxolol (0.04) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,12) = 0.3$, $p > .05$; ICI118,551 (10.0) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,16) = 25.0$, $p < .01$; ICI118,551 (0.63) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,14) = 5.5$, $p < .05$; and ICI118,551 (0.04) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,12) = 0.3$, $p > .05$. *NAD*: vehicle + (-)-pindolol vs. vehicle + vehicle, $F(1,17) = 41.7$, $p < .01$; betaxolol (10.0) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,16) = 12.3$, $p < 0.01$; betaxolol (0.63) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,14) = 0.4$, $p > .05$; betaxolol (0.04) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,13) = 0.1$, $p > .05$; ICI118,551 (10.0) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,16) = 5.7$, $p < .05$; ICI118,551 (0.63) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,14) = 0.2$, $p > .05$; and ICI118,551 (0.04) + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,12) = 0.1$, $p > .05$. Asterisks indicate significance of differences of betaxolol + (-)-pindolol and ICI118551 + (-)-pindolol to vehicle + (-)-pindolol vehicle values. * $p < .05$.

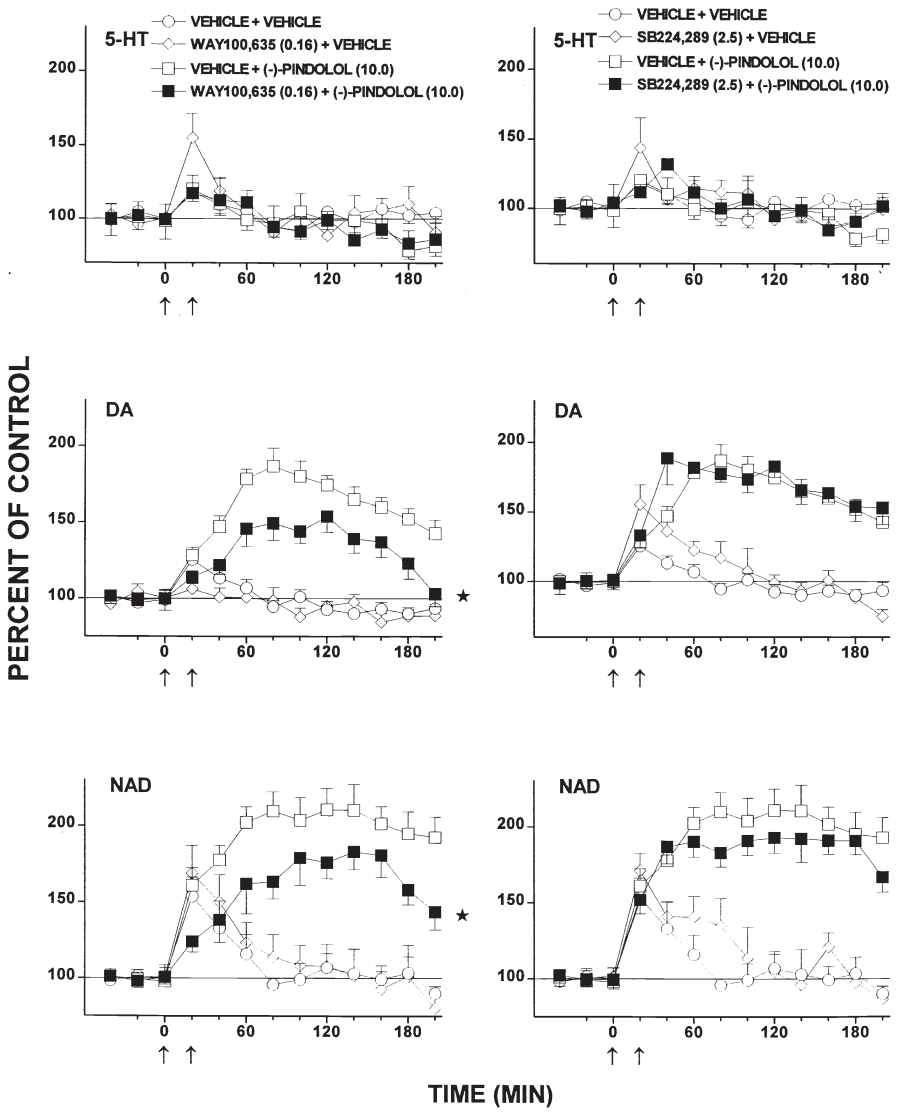

Figure 4. Influence of the 5-HT_{1A} antagonist, WAY100,635 and of the 5-HT_{1B} antagonist, SB224,289, upon modulation of FCX levels of DA, and NAD as compared to 5-HT by (-)-pindolol. Data are means ± S.E.M.s of 5-HT, DA, and NAD levels expressed as a percentage of basal pre-injection values (= 100%). N = 5–13 per value. ANOVAs were performed over 40–200 min. *5-HT*: WAY100,635 + vehicle vs. vehicle + vehicle, $F(1,20) = 0.1$, $p > .05$; WAY100,635 + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,13) = 0.1$, $p > .05$; SB224,289 + vehicle vs. vehicle + vehicle, $F(1,17) = 0.1$, $p > .05$; and SB224,289 + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,11) = 1.3$, $p > .05$. *DA*: WAY100,635 + vehicle vs. vehicle + vehicle, $F(1,19) = 0.9$, $p > .05$; WAY100,635 + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,16) = 13.8$, $p < .01$; SB224,289 + vehicle vs. vehicle + vehicle, $F(1,16) = 1.5$, $p > .05$; and SB224,289 + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,13) = 0.8$, $p > .05$. *NAD*: WAY100,635 + vehicle vs. vehicle + vehicle, $F(1,17) = 0.1$, $p > .05$; WAY100,635 + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,16) = 5.0$, $p < .05$; SB224,289 + vehicle vs. vehicle + vehicle, $F(1,13) = 0.8$, $p > .05$; and SB224,289 + (-)-pindolol vs. vehicle + (-)-pindolol, $F(1,13) = 0.9$, $p > .05$. Asterisks indicate significance of differences of WAY100,635 + (-)-pindolol to vehicle + (-)-pindolol vehicle values. * $p < .05$. Influence of vehicle + (-)-pindolol vs. vehicle + vehicle see Figure 3.

tine upon NAD levels was *not* greater in the presence of (-)-pindolol (Figure 5). Rather, the effect of (-)-pindolol plus fluoxetine, and of (-)-pindolol plus duloxetine, upon NAD levels, was equivalent to that of fluoxetine and duloxetine, alone, respectively (Figure 5).

Influence of Betaxolol and ICI118,151 upon the Actions of Fluoxetine

As shown in Figure 6, neither betaxolol (10.0) nor ICI118,551 (10.0) modified the influence of fluoxetine (10.0) upon dialysate levels of DA, NAD, or 5-HT in

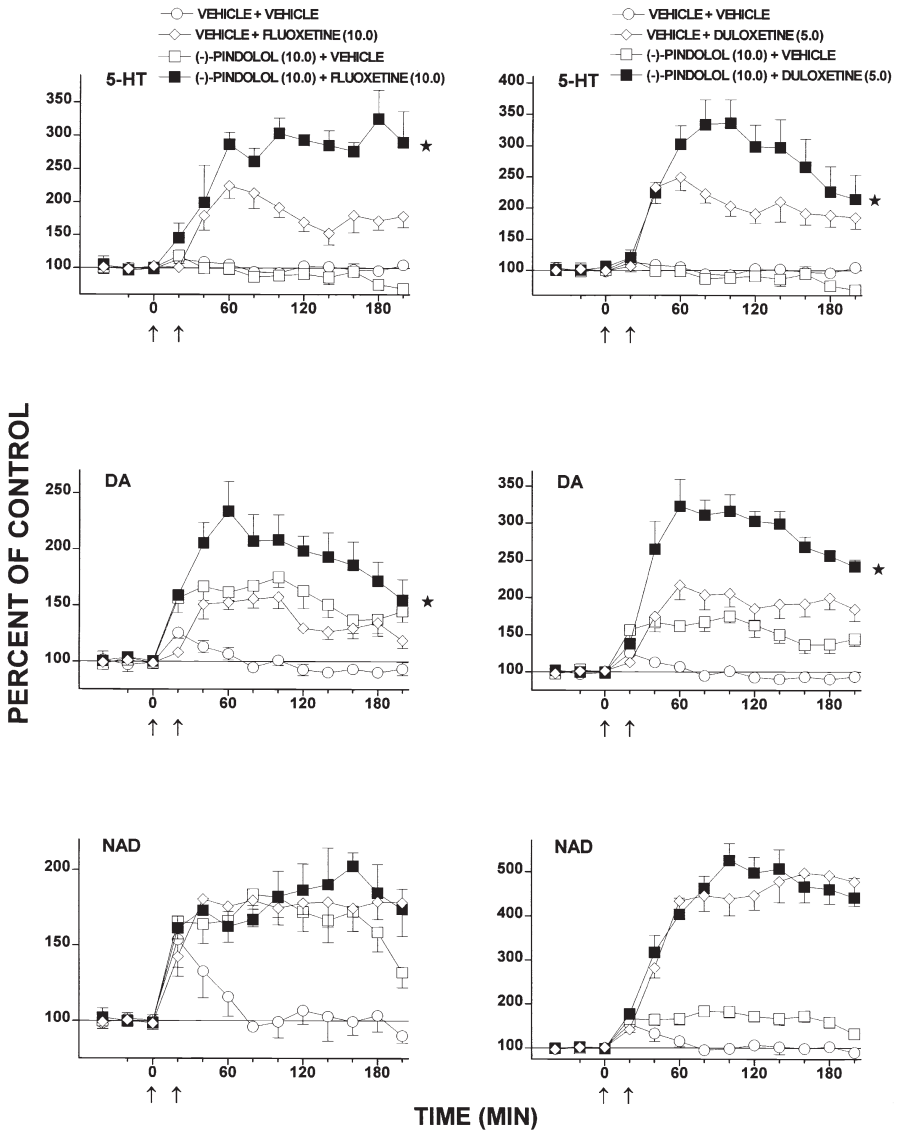

Figure 5. Influence of (-)-pindolol upon modulation of FCX levels of DA, and NAD as compared to 5-HT by fluoxetine and duloxetine. Data are means ± S.E.M.s of 5-HT, DA, and NAD levels expressed as a percentage of basal pre-injection values (= 100%). N = 5–12 per value. ANOVAs were performed over 40–200 min. *5-HT*: vehicle + fluoxetine vs. vehicle + vehicle, $F(1,21) = 45.0$, $p < .01$; (-)-pindolol + fluoxetine vs. vehicle + fluoxetine, $F(1,15) = 18.9$, $p < .01$; vehicle + duloxetine vs. vehicle + vehicle, $F(1,21) = 44.2$, $p < .01$; and (-)-pindolol + duloxetine vs. vehicle + duloxetine, $F(1,17) = 6.2$, $p < .05$. *DA*: vehicle + fluoxetine vs. vehicle + vehicle, $F(1,20) = 39.4$, $p < .01$; (-)-pindolol + fluoxetine vs. vehicle + fluoxetine, $F(1,16) = 16.4$, $p < .01$; vehicle + duloxetine vs. vehicle + vehicle, $F(1,20) = 30.1$, $p < .01$; and (-)-pindolol + duloxetine vs. vehicle + duloxetine, $F(1,18) = 16.2$, $p < .01$. *NAD*: vehicle + fluoxetine vs. vehicle + vehicle, $F(1,19) = 21.2$, $p < .01$; (-)-pindolol + fluoxetine vs. vehicle + fluoxetine, $F(1,16) = 0.1$, $p > .05$; vehicle + duloxetine vs. vehicle + vehicle, $F(1,19) = 55.1$, $p < .01$; and (-)-pindolol + duloxetine vs. vehicle + duloxetine, $F(1,18) = 0.1$, $p > .05$. Asterisks indicate significance of differences of (-)-pindolol + fluoxetine and (-)-pindolol + duloxetine to vehicle + fluoxetine and to vehicle + duloxetine values, respectively. * $p < .05$. Influence of (-)-pindolol + vehicle vs. vehicle + vehicle see Figure 2.

FCX. Administered alone, ICI118,551 (10.0) did not alter dialysate levels of DA, NAD, or 5-HT (Figure 6). Furthermore, betaxolol (10.0) did not modify dialysate levels of NAD and 5-HT, yet slightly decreased those of DA (Figure 6).

Influence of WAY100,635 and SB224,289 upon the Actions of Fluoxetine

WAY100,635 (0.16) potentiated the influence of fluoxetine (10.0) upon dialysate levels of 5-HT without modifying its influence upon levels of DA or NAD (Figure 7).

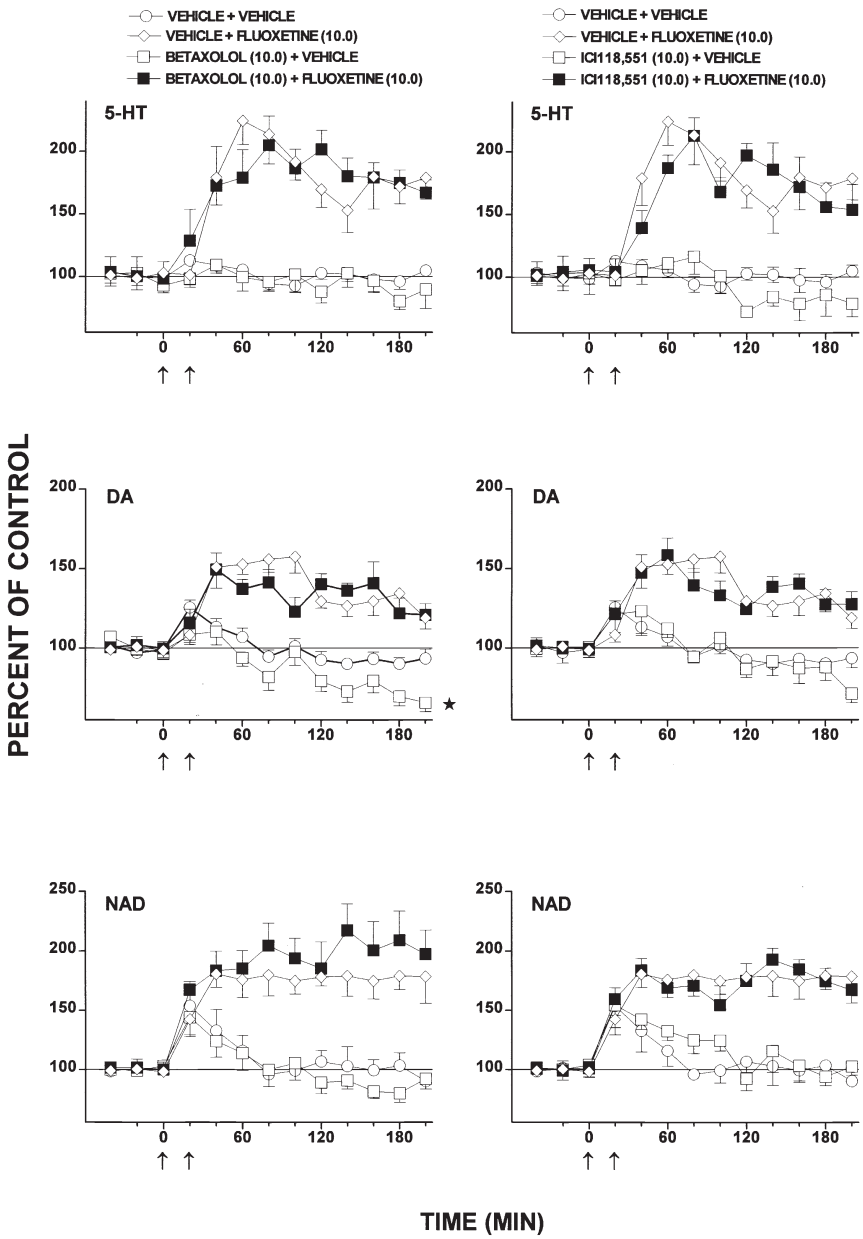

Figure 6. Influence of the β_1- and β_2- AR antagonists, betaxolol and ICI118,551, respectively, upon modulation of FCX levels of DA, and NAD as compared to 5-HT by fluoxetine. Data are means ± S.E.M.s of 5-HT, DA, and NAD levels expressed as a percentage of basal pre-injection values (= 100%). N = 6–12 per value. ANOVAs were performed over 40–200 min. *5-HT*: betaxolol (10.0) + fluoxetine vs. vehicle + fluoxetine, $F(1,16) = 0.1$, $p > .05$; betaxolol (10.0) + vehicle vs. vehicle + vehicle, $F(1,17) = 0.5$, $p > .05$; ICI118,551 (10.0) + fluoxetine vs. vehicle + fluoxetine, $F(1,16) = 0.3$, $p > .05$; and ICI118,551 (10.0) + vehicle vs. vehicle + vehicle, $F(1,16) = 1.1$, $p > .05$. *DA*: betaxolol (10.0) + fluoxetine vs. vehicle + fluoxetine, $F(1,17) = 0.4$, $p > .05$; betaxolol (10.0) + vehicle vs. vehicle + vehicle, $F(1,16) = 5.5$, $p < .05$; ICI118,551 (10.0) + fluoxetine vs. vehicle + fluoxetine, $F(1,17) = 0.1$, $p > .05$; and ICI118,551 (10.0) + vehicle vs. vehicle + vehicle, $F(1,15) = 0.1$, $p > .05$. *NAD*: betaxolol (10.0) + fluoxetine vs. vehicle + fluoxetine, $F(1,17) = 0.9$, $p > .05$; betaxolol (10.0) + vehicle vs. vehicle + vehicle, $F(1,14) = 0.5$, $p > .05$; ICI118,551 (10.0) + fluoxetine vs. vehicle + fluoxetine, $F(1,16) = 0.1$, $p > .05$; and ICI118,551 (10.0) + vehicle vs. vehicle + vehicle, $F(1,14) = 1.0$, $p > .05$. Influence of vehicle + fluoxetine vs. vehicle + vehicle see Figure 5.

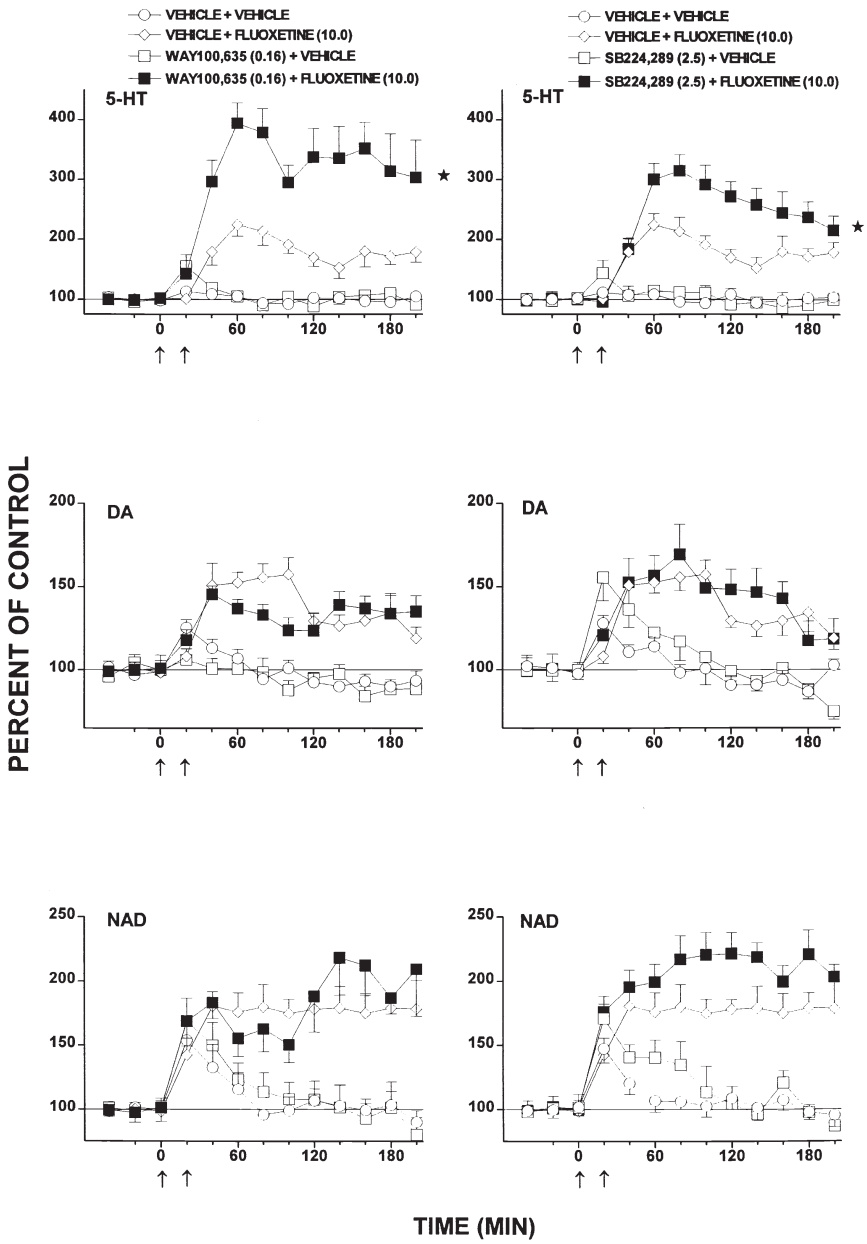

Figure 7. Influence of the 5-HT_{1A} antagonist, WAY100,635, and of the 5-HT_{1B} antagonist, SB224,289, upon modulation of FCX levels of DA, and NAD as compared to 5-HT by fluoxetine. Data are means ± S.E.M.s of 5-HT, DA, and NAD levels expressed as a percentage of basal pre-injection values (= 100%). N = 6–12 per value. ANOVAs were performed over 40–200 min. *5-HT*: WAY100,635 + fluoxetine vs. vehicle + fluoxetine, $F(1,17) = 20.7$, $p < .01$; and SB224,289 + fluoxetine vs. vehicle + fluoxetine, $F(1,16) = 11.1$, $p < .01$. *DA*: WAY100,635 + fluoxetine vs. vehicle + fluoxetine, $F(1,17) = 0.4$, $p > .05$; and SB224,289 + fluoxetine vs. vehicle + fluoxetine, $F(1,16) = 0.2$, $p > .05$. *NAD*: WAY100,635 + fluoxetine vs. vehicle + fluoxetine, $F(1,17) = 0.1$, $p > .05$; and SB224,289 + fluoxetine vs. vehicle + fluoxetine, $F(1,16) = 2.9$, $p > .05$. Asterisks indicate significance of differences of WAY100,635 + fluoxetine and SB224,289 + fluoxetine and to vehicle + fluoxetine values. * $p < .05$. Influence of vehicle + fluoxetine, WAY100,635 + vehicle, and SB224,289 + vehicle vs. vehicle + vehicle see Figures 4 and 5.

SB224,289 (2.5) acted similarly in facilitating the increase in 5-HT levels elicited by fluoxetine without significantly modifying its influence upon levels of DA or NAD (Figure 7).

DISCUSSION

Interaction of (-)-Pindolol with 5-HT_{1A} and 5-HT_{1B} Autoreceptors

(-)-Pindolol has been shown to display agonist or antagonist properties at various populations of 5-HT_{1A} recep-

tors and, notably, it acts as a partial agonist at cloned, human 5-HT_{1A} receptors (see below). At these sites, its efficacy is *less* than that of 5-HT and 8-OH-DPAT, yet *greater* than that of WAY100,635 (Meltzer and Maes 1996; Newman-Tancredi et al. 1998; Wolff and Leander 1997). This suggests that (-)-pindolol may enhance or diminish activity at 5-HT_{1A} sites dependent upon the concentration of 5-HT available. This observation may, thus, underlie the continuing discussion as to whether (-)-pindolol behaves as an "antagonist" or "agonist" at raphe-localized 5-HT_{1A} autoreceptors in rats (Artigas et al. 1996; Clifford et al. 1998; Corradetti et al. 1998; Fornal et al. 1998; Romero et al. 1996; Hjorth and Sharp 1993). In our studies, we have observed that (-)-pindolol behaves as a partial agonist in *sub*maximally inhibiting the firing rate of dorsal raphe nucleus neurones (Lejeune and Millan, unpublished observations). It might be argued that such a partial agonist action of (-)-pindolol at 5-HT_{1A} autoreceptors is inconsistent with its minimal suppressive influence upon FCX levels of 5-HT seen herein and in other studies (Assie and Koek 1996; Hjorth and Sharp 1993; Sharp and Hjorth 1990). However, 1) the firing rate of dorsal raphe nucleus neurones is *not* invariably reflected in release at the terminal level (Davidson and Stamford 1998); 2) there are regional differences in the effects of 5-HT_{1A} autoreceptor stimulation upon postsynaptic levels of 5-HT (Invernizzi et al. 1997; Kreiss and Lucki 1997; Malagié et al. 1996; Romero and Artigas 1997); and 3) antagonist actions at 5-HT_{1B} terminals (see below) may mask its partial agonist actions at raphe-localized 5-HT_{1A} receptors (Assie and Koek 1996). In any case, the *antagonist* actions of (-)-pindolol at 5-HT_{1A} autoreceptors clearly prevailed in its antagonism of the inhibitory influence of 8-OH-DPAT upon FCX levels of 5-HT seen herein and elsewhere (Sharp and Hjorth 1990) (Figure 2). This finding is analogous to that described for the selective and neutral 5-HT_{1A} antagonist, WAY100,635 (Gobert et al. 1998) (see Results).

(-)-Pindolol also shows marked affinity for rat 5-HT_{1B} receptors and, at these sites, there is a consensus that (-)-pindolol exerts antagonist properties (Adham et al. 1992; Assie and Koek 1996; Bourin et al. 1998; Hoyer and Schoeffter 1991). Indeed, in line with this notion, in analogy to the selective 5-HT_{1B} antagonist, SB224,289, (-)-pindolol blocked the decrease in FCX levels elicited by the 5-HT_{1B} agonist, GR46611 (Gobert et al. 1998; Millan et al. in press).

Modulation by (-)-Pindolol of Dialysate Levels of DA and NAD in FCX: Potential Role of β-ARs

In contrast to (-)-pindolol, which dose-dependently increased FCX levels of DA and NAD, the preferential β_1- and β_2-AR antagonists, betaxolol and ICI118,551, respectively (Conway et al. 1987; Tsuchihashi et al. 1990), did not mimic this action. This difference suggests that the partial *agonist* actions of (-)-pindolol at β_1- and/or β_2-ARs may be involved in its distinctive actions (Frishman 1983; Haeusler 1990; Hoffman and Lefkowitz 1996; Kaur and Ahlenius 1997). Several other lines of evidence may be evoked.

First, as concerns NAD, the preferential β_2-AR agonist, clenbuterol, (O'Donnell 1997), elevates DA and NAD levels in FCX (Lejeune et al. 1998). *Second*, β-AR agonists enhance the release of NAD from adrenergic terminals in the cerebral cortex and hypothalamus *in vitro* (Misu and Kubo 1986; Murugaiah and O'Donnell 1995). In these studies, β-AR antagonists blocked the actions of β-AR agonists but were inactive alone suggesting a lack of tonic control of NAD release.

Indeed, betaxolol and ICI118,551 did not decrease NAD levels herein. *Third*, β-AR agonists accelerate NAD turnover in the cerebral cortex and hypothalamus (O'Donnell 1993). *Fourth*, herein, betaxolol *and* ICI118,551 both inhibited the influence of (-)-pindolol upon FCX dialysate levels of DA and NAD (Figure 3). This provides an interesting analogy to the *in vitro* studies of Murugaiah and O'Donnell (1995), in which selective antagonists at both β_1- *and* β_2-ARs inhibited stimulation of NAD release by β-AR agonists in the cortex. Thus, both β_1- and β_2-ARs might be involved in this action, in line with reports that they each are involved in the AD effects of β-AR agonists (Martin et al. 1986; O'Donnell 1997; O'Donnell et al. 1994). In fact, in both the present study and that of Murugaiah and O'Donnell (1995), antagonists at β_1- and β_2-ARs in each case inhibited the influence of (-)-pindolol upon DA levels by *more* than 50 %. The reason for this unclear. There may exist some redundancy in their roles, or they may interact in the expression of their actions. Alternatively, it is possible that the β_1- and β_2-AR antagonists employed are not absolutely selective: that is, they may also partially block β_2- and β_1-AR receptors, respectively.

Although the concentration of β_2-ARs in the FCX is modest (Nicholas et al. 1993), they may be preferentially localized on the terminals of adrenergic neurones, in analogy to the peripheral sympathetic system (Misu and Kubo 1986). As concerns β_1-ARs, which are concentrated in the FCX, a subpopulation may, in analogy to peripheral adrenergic nerves likewise be localized on the terminals of adrenergic fibres therein (Misu and Kubo 1986). Nevertheless, the neuronal localization of β_1-and β_2-ARs modulating NAD release is unclear, and indirect actions are possible. For example, β-ARs enhance frontocortical release of glutamate which may, subsequently, facilitate NAD release via excitatory NMDA and/or AMPA receptors (Herrero and Sánchez-Prieto 1996; Ikegaya et al. 1997; Ruzicka and Jhamandas 1993).

Although, in an *in vitro* study, activation of β-ARs facilitated the outflow of DA in the striatum and hypothalamus, there is no information concerning such a

possible influence of β-ARs in the FCX (see Misu and Kubo 1986). Further, β-AR ligands do not modulate the electrical activity of ventrotegmental area (VTA)-localized, dopaminergic cell bodies (Grenhoff et al. 1993) (Lejeune and Millan, unpublished observations). However, the VTA contains mRNA encoding β_1-ARs which may be transported to dopaminergic terminals in the FCX. Therein, both direct and/or indirect actions at β_1- and/or β_2-ARs may be implicated in the increase in extracellular DA levels (Nicholas et al. 1993).

(-)-Pindolol shows low affinity and efficacy at rat β_3-ARs, the presence of which in mature, rodent CNS remains unclear (Summers et al. 1995). Thus, a role of β_3-ARs in the actions of (-)-pindolol is unlikely.

Modulation by (-)-Pindolol of Dialysate Levels of DA and NAD in FCX: Potential Role of 5-HT_{1A} and 5-HT_{1B} Receptors

It is well-established that 5-HT_{1A} receptor agonists enhance the activity of frontocortical dopaminergic pathways (Gobert et al. 1998; Lejeune et al. 1997, 1998; Millan et al. 1997) (Figure 2). One possible underlying mechanism (Lejeune et al. 1997; Lejeune and Millan 1998) is a role of 5-HT_{1A} autoreceptors, the activation of which may disinhibit mesocortical dopaminergic pathways by removing a suppressive influence of 5-HT exerted via excitatory 5-HT_{2C} receptors localized on GABAergic interneurones in the VTA (Millan et al. 1998b; Prisco et al. 1994). A similar mechanism may intervene in the elevation of FCX levels of NAD by 5-HT_{1A} receptor agonists (Done and Sharp 1994; Gobert et al. 1998). Alternatively, postsynaptic 5-HT_{1A} receptors localized on dopaminergic and adrenergic neurones in the VTA and locus coeruleus, respectively, may be involved (Done and Sharp 1994; Lejeune et al. 1997; Lejeune and Millan 1998). Thus, the partial agonist actions of (-)-pindolol at 5-HT_{1A} sites may contribute to its increase in FCX levels of DA and NAD and, in line with this possibility, its action was partially attenuated by WAY100,635. Inasmuch as (-)-pindolol *little* influenced 5-HT levels alone, the population of 5-HT_{1A} receptors involved is likely to be *post*synaptic and, in this light, it is intriguing to note that (-)-pindolol may behave as an agonist or antagonist at 5-HT_{1A} receptors in various structures—an issue as yet to be addressed for the VTA and locus coeruleus (Corradetti et al. 1998; Romero et al. 1996).

As discussed above, (-)-pindolol shows marked activity at 5-HT_{1B} receptors, two populations of which may potentially modify DA and NAD levels in FCX. *First*, 5-HT_{1B} receptors inhibitory to VTA-localized GABAergic interneurones (Kelland and Chiodo 1996); and *second*, inhibitory 5-HT_{1B} receptors on serotonergic terminals themselves. However, (-)-pindolol is a pure antagonist at 5-HT_{1B} receptors (Adham et al. 1992; Assie and Koek 1996; Hoyer and Schoeffter 1991). Furthermore, the selective 5-HT_{1B} antagonist, SB224,289, did *not* affect the increase in FCX levels of DA or NAD elicited by (-)-pindolol. Thus, stimulation of 5-HT_{1B} receptors is unlikely to be involved in the increase in FCX levels of DA and NAD elicited by (-)-pindolol.

Modulation by (-)-Pindolol of the Influence of SSRIs upon FCX Levels of 5-HT, DA, and NAD

In line with previous studies (see Gobert et al. 1997b; Tanda et al. 1994), fluoxetine and duloxetine elevated FCX levels of NAD and DA. While their relative influence upon NAD levels may, at least partially, reflect their actions at NAD reuptake sites, respectively, their enhancement in DA levels possibly involves an action of 5-HT at excitatory 5-HT_3 receptors on dopaminergic terminals (Tanda et al. 1995).

The present study extends previous work (Artigas et al. 1996; Gartside et al. 1995; Hjorth 1996) in showing that, in freely-moving rats, (-)-pindolol facilitates the elevation of 5-HT levels in the FCX elicited by fluoxetine and duloxetine. In addition, (-)-pindolol enhanced (in an apparently additive fashion) the influence of SSRIs upon FCX levels of DA, but *not* NAD. Thus, the nature of the interaction of (-)-pindolol with fluoxetine and duloxetine was different for 5-HT and DA versus NAD in the same dialysate samples, suggesting that pharmacokinetic factors are very unlikely to be involved in these effect (see also Artigas et al. 1996).

Potentiation by (-)-Pindolol of SSRI-Induced Increases in 5-HT Levels: Underlying Mechanisms

First, a role of β-ARs is unlikely since acute administration of β-AR agonists and antagonists does not modify, either alone or upon co-administration with fluoxetine, serotonergic transmission (Figure 6) (Bouthillier et al. 1991; Hjorth et al. 1996; Lejeune et al. 1998) (Gobert A, unpublished observations). Furthermore, there is no obvious neuroanatomical basis for a modulation of serotonergic transmission by β-ARs (Nicholas et al. 1993). *Second*, WAY100,635 mimicked the facilitatory influence of (-)-pindolol upon the fluoxetine-evoked increase in FCX levels of 5-HT (Gartside et al. 1995; Gobert et al. 1997b) suggesting that a reduction by (-)-pindolol of activity at 5-HT_{1A} autoreceptors might be involved. It might be argued that this would be incompatible with intrinsic partial agonist actions of (-)-pindolol at 5-HT_{1A} autoreceptors (Clifford et al. 1998) (see above) since 5-HT_{1A} agonists inhibit the influence of SSRIs upon FCX levels of 5-HT (Dawson and Nguyen 1998; Gobert et al. 1997a; Hjorth 1996). However, as emphasized above, (-)-pindolol possesses *lower* efficacy than 5-HT at 5-HT_{1A} receptors (Newman-Tancredi et al. 1998). It may, thus, in analogy to its antagonist action versus 8-OH-DPAT,

reduce the activation of raphe-localized 5-HT_{1A} autoreceptors by 5-HT following fluoxetine administration. A *third* mechanism is offered by the antagonist actions of (-)-pindolol at 5-HT_{1B} autoreceptors localized on serotonergic terminals - and, possibly, cell bodies (Bourin et al. 1998; Davidson and Stamford 1995; Hoyer and Schoeffter 1991) (Figure 2). Indeed, the 5-$HT_{1B/1D}$ antagonists, GR127,935 and SB224,289 (Figure 7), markedly facilitate the increase in FCX levels of 5-HT elicited by SSRIs (Davidson and Stamford 1997; Gaster et al. 1998; Gobert et al. 1997b; Sharp et al. 1997).

Influence of (-)-Pindolol upon SSRI-Induced Increases in DA and NAD Levels: Underlying Mechanisms

As discussed above, different mechanisms underlie the induction of FCX levels of DA by SSRIs versus (-)-pindolol and a parsimonious interpretation of their apparently additive effects is that they represents the sum of their individual actions. Consistent with this possibility, the 5-HT_{1A} autoreceptor agonists, buspirone and 8-OH-DPAT, exert an apparently additive influence with fluoxetine upon FCX levels of DA (Gobert et al. 1997a). The contrasting *lack* of additive effects of SSRIs and (-)-pindolol upon NAD levels was surprising. Nevertheless, in analogy, buspirone and 8-OH-DPAT similarly do *not* additively increase FCX levels of NAD with SSRIs (Gobert et al., in press).

GENERAL DISCUSSION: RELEVANCE TO THE TREATMENT OF DEPRESSIVE STATES AND THE ACTIONS OF ADS

First, the present data are consistent with the hypothesis that, following SSRI administration, (-)-pindolol may attenuate stimulation of 5-HT_{1A} autoreceptors by 5-HT and thereby increase postsynaptic 5-HT levels (Artigas et al. 1996). However, blockade of 5-HT_{1B} receptors may *also* be involved in this effect in the rat (Hoyer and Schoeffter 1991), a crucial point inasmuch as (-)-pindolol has *no* affinity for homologous human 5-HT_{1B} receptors (Adham et al. 1992). *Second*, upon administration *alone*, (-)-pindolol elevates extracellular level of DA and NAD, but *not* 5-HT, in the FCX. Furthermore, in the presence of (-)-pindolol, the influence of SSRIs upon FCX levels of DA is more pronounced. Both frontocortical dopaminergic and adrenergic pathways control mood and cognition and a deficit in mesocortical dopaminergic transmission is implicated in depressive states (Caldecott-Hazard et al. 1991; Zacharko and Anisman 1991). Moreover, a potentiation of DA levels in the FCX may be a common denominator for many AD agents (Rivet et al. 1998; Tanda et al. 1994). The present findings add, then, a novel dimension to the interpretation of clinical observations of the adjunctive treatment of depression with AD agents plus (-)-pindolol: dopaminergic (and adrenergic) as well as serotonergic mechanisms may be involved (Artigas et al. 1996; Berman et al. 1997; McAskill et al. 1998). A further implication of the present data is that the effects of (-)-pindolol *alone* in depressed patients require evaluation. *Third*, the elevation by (-)-pindolol of FCX levels of DA and NAD may involve its partial agonist actions at 5-HT_{1A} autoreceptors, but its partial agonist actions at $\beta_{1/2}$-ARs are more strongly implicated. These findings are of relevance to experimental and clinical evidence that stimulation of β-ARs is associated with AD properties, whereas a commonly-reported side-effect of β-AR antagonists—employed for the treatment of hypertension—is depression (O'Donnell et al. 1994; Thiessen et al. 1990; Zohar et al. 1987). Furthermore, several classes of AD agent downregulate β-ARs (principally β_1) in the FCX and certain other brain regions (Duncan et al. 1994; Hosoda and Duman 1993; Okada and Tokumitsu 1994). Nevertheless, rigourous, controlled clinical studies of the influence of β-AR stimulation and blockade upon mood are awaited. *Fourth*, (-)-pindolol is *not* an ideal tool for an evaluation of the 5-HT_{1A} autoreceptor antagonist-desensitization hypothesis inasmuch as it behaves as a *partial* agonist at 5-HT_{1A} autoreceptors and β-ARs, as well as an antagonist at (rat) 5-HT_{1B} receptors. Further, via actions at these sites, it enhances FCX levels of DA and NAD upon administration alone. On the other hand, these actions of (-)-pindolol suggest, on empirical grounds, that it may be a particularly useful adjunctive agent for an improvement in AD effect. *Fifth*, certain rodent-human differences should be mentioned. Notably, the above-mentioned absence of affinity of (-)-pindolol for h5-HT_{1B} receptors, yet its significant affinity for human versus rat β_3-ARs, which have been implicated in depressive states (Adham et al. 1992; Liggett 1992; Simiand et al. 1992).

Several limitations of the present study should be noted. The present results were obtained employing single doses of (-)-pindolol in interaction with single doses of fluoxetine and duloxetine. It would, thus, be interesting to examine the actions of other doses of (-)-pindolol and SSRIs. However, the performance of complete dose-response curves for *both* interacting drugs would be, in principle, necessary in order to permit the formal demonstration of "additivity" or "synergy" of drug effects via isobolographic analysis. Such studies would be difficult to perform and prohibitively time-consuming for the dialysis approach adopted herein. Thus, the magnitude of the mutual facilitatory influence of (-)-pindolol and SSRIs upon DA levels is difficult to precisely define. A further point to emphasize is that the present studies were undertaken in the FCX. While this region is involved in depressive states (Goodwin 1997; Zacharko and Anisman 1991), and al-

Table 2. Summary of Actions of (-)-Pindolol in Comparison to Those of Selective Ligands at Receptor Types with Which it Interacts in the Rat

		Vehicle			+ Fluoxetine (duloxetine)		
Drug	**Class**	**5-HT**	**DA**	**NAD**	**5-HT**	**DA**	**NAD**
Vehicle		**0**	**0**	**0**	↑	↑	↑
(-)-Pindolol	Mixed[a]	0	↑	↑	↑↑	↑↑	↑
8-OH-DPAT	5-HT_{1A} ago[b]	↓	↑	↑	0	↑↑	↑
WAY100,635	5-HT_{1A} ant	0	0	0	↑↑	↑	↑
SB224,289	5-HT_{1B} ant	0	0	0	↑↑	↑	↑
Betaxolol	β_1 ant	0	↓	0	↑	↑	↑
ICI118,551	β_2 ant	0	0	0	↑	↑	↑

↑: increase; ↓: decrease and ↑↑: apparent additive influence. [a](-)-Pindolol acts as a 5-HT_{1A} partial agonist and as an antagonist at 5-HT_{1B}, β_1- and β_2-adrenergic receptors (see text); [b]see Gobert et al. Neuroscience, in press.
Ago = agonist; ant = antagonist.

lows for the simultaneous determination of 5-HT, DA, and NAD levels (Gobert et al. 1998), it would be of interest to extend the present work to other corticolimbic structures, such as the hippocampus and septum, likewise implicated in the actions of SSRIs and other classes of AD agent (Maes and Meltzer 1995).

CONCLUSIONS

To summarize (see Table 2), the influence of (-)-pindolol upon monoaminergic transmission and depressive states is *not* restricted to an enhancement in the influence of SSRIs upon postsynaptic 5-HT levels via the blockade of 5-HT_{1A} autoreceptors: 5-HT_{1B} receptor blockade may also be involved in this action. Moreover, (-)-pindolol exerts an apparently additive, facilitatory influence with SSRIs upon FCX levels of *DA*. Most intriguingly, the partial agonist actions of (-)-pindolol at $\beta_{1/2}$-ARs and, though less markedly at 5-HT_{1A} receptors, contribute to its *intrinsic* facilitatory influence upon FCX levels of DA and NAD. These data raise the possibility that (-)-pindolol *itself* may modify the emotional and cognitive deficits of depressive states. In conclusion, then, both alone and in interaction with SSRIs, (-)-pindolol elicits a highly complex pattern of effects upon dopaminergic, adrenergic, and serotonergic transmission via 5-HT_{1A}, 5-HT_{1B} receptors and $\beta_{1/2}$-ARs. These findings may be of considerable significance to our understanding of the influence of (-)-pindolol upon depressive states and the actions of AD agents.

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