Effects of Immobilization Stress and of a Benzodiazepine Derivative on Rat Central Dopamine System

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The effects of a novel benzodiazepine derivative, Ro 16-6028 on rat brain dopamine system were examined under stress and non-stress conditions. Thirty minutes restraint stress increased dopamine synthesis in two dopamine neuron regions, prefrontal cortex and nucleus accumbence. Ro 16-6028 inhibited potently dopamine synthesis in prefrontal cortex, nucleus accumbens and striatum in dose dependent manner under non-stress condition. Furthermore, Ro 16-6028 reverses the stress-induced augmentation of the synthesis in prefrontal cortex. These findings indicate that Ro 16-6028 has an anxiolytic profile and that central dopamine system plays an important role in stress reaction. (Key words: stress, dopamine synthesis, tyrosine hydroxylase, benzodiazepine)

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Benzodiazepine (BDZ) derivatives are commonly prescribed for preanesthetic medication and to supplement or to induce and maintain anesthesia. Recent studies have shown that BDZ agonists decrease dopamine (DA) turnover in mesotelencephalic DA neurons, and prevent stress-induced augmentation of DA turnover in prefrontal cortex[1–3].

The pharmacological properties, however, are not the same among their derivatives. Ro 16-6028, a novel BDZ partial agonist[4], possesses the anticonvulsant and anxiolytic actions of typical BDZ agonists, but exhibits much less muscle relaxant and sedative properties relative to full agonists[5]. We therefore examined the effect of Ro 16-6028 on *in vivo* tyrosine hydroxylase (TH) activity in various brain regions as an index of DA synthesis.

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Material and Methods

Male Sprague-Dawley rats (250–300g) were used as subjects. TH activity was evaluated as DOPA accumulation following administration of I-aromatic amino acid decarboxylase inhibitor, NSD-1015. Animals received NSD-1015 (100 $mg \cdot kg^{-1}$) and various doses of Ro 16-6028 intraperitoneally 35 and 30 min before sacrifice, respectively. After decapitation brains were rapidly removed, and the following regions were dissected out from 1 mm-thick coronal slices; anteromedial prefrontal cortex (PFC), nucleus accumbens (NAS), nucleus caudateputamen

Table 1. Effect of Ro 16-6028 on *in vivo* TH activity in various regions

Dose $mg \cdot kg^{-1}$	PFC	NAC	CP	SN	VTA	RRF
0	2.95 ± 0.27 (100)	32.46 ± 1.10 (100)	28.73 ± 1.20 (100)	8.28 ± 0.50 (100)	41.16 ± 3.13 (100)	4.74 ± 0.67 (100)
0.2	2.18 ± 0.18 (73.8)*	27.30 ± 1.50 (84.1)*	22.73 ± 1.49 (79.1)*	7.51 ± 0.54 (90.7)	36.39 ± 2.78 (88.4)*	4.73 ± 0.61 (99.9)
0.5	2.73 ± 0.11 (80.3)**	26.25 ± 1.12 (80.9)**	22.69 ± 0.82 (79.0)**	6.75 ± 0.52 (81.5)*	29.87 ± 2.26 (72.6)**	4.49 ± 0.36 (94.6)
1.0	2.13 ± 0.21 (72.2)**	21.42 ± 2.58 (66.0)**	27.08 ± 1.50 (94.3)	6.50 ± 0.75 (78.6)*	24.21 ± 3.23 (58.8)**	5.21 ± 0.55 (109.9)
5.0	2.63 ± 0.33 (89.2)	26.61 ± 1.86 (82.0)*	27.65 ± 1.47 (96.2)	6.92 ± 0.25 (83.6)*	33.50 ± 1.76 (81.4)*	5.15 ± 0.97 (108.7)

*$P < 0.05$, **$P < 0.01$; significantly different from control.

NSD-1015 (100 $mg \cdot kg^{-1}$) and various doses of Ro 16-6028 were injected 35 and 30 min before sacrifice, respectively. Each value indicates mean ± standard error (ng/mg protein/35 min) from 4–6 animals. The numbers in parentheses show percent of control value.

(CP), substantia nigra (SN), ventral tegmental area (VTA) and retrorubral field (RRF) as previously described[6]. The last three areas correspond to the A_{10}, A_9, and A_8 DA cell groups, respectively. Tissues were stored at −70°C until assayed for DOPA by HPLC with double ECD electrodes (model 5100, ESA Inc. Bedford, MA) using α-methyl DOPA as an internal standard[7]. Protein amounts were quantified using a dye reagent (Bio-Rad Labs, Richmond, CA).

To determine the effect of Ro 16-6028 on stress-induced activation of TH, animals were subjected to restraint stress. NSD-1015 (100 $mg \cdot kg^{-1}$) were injected 5 min after Ro 16-6028 (0.5 $mg \cdot kg^{-1}$) or vehicle administration, then immobilized in a wire mesh restraint apparatus for 30 min at room temperature. Non stress-treated rats were left in their home cages and separated from stress-treated groups. Animals were then sacrificed and regional DOPA levels were measured as described above. The data were analyzed by means of ANOVA and Bonferroni t-test as post-hoc analyses as indicated.

Ro 16-6028 was a generous gift from Prof. W. Haefely of F. Hoffmam- La Roche & Co., Basle, Switzerland. Ro 16-6028 was suspended in physiological saline with one drop (appx. 50 μl) of Tween 80 per 10 ml saline. NSD-1015 (m-hydroxybenzylhydrazine) and Tween 80 were purchased from Sigma, St. Louis, MO.

Results

Ro 16-6028 inhibited potently *in vivo* TH activity in mesocortical (PFC), mesolimbic (NAS) and striatal (CP) DA system terminal field regions (table 1, fig. 1). The enzyme activity in two DA cell regions (SN and VTA) was also decreased by Ro 16-6028. The inhibition of TH activity was observed in all terminal regions at a dose of 0.2 $mg \cdot kg^{-1}$, however, at the highest dose tested (5 $mg \cdot kg^{-1}$), the activity was not reduced significantly in either PFC or CP. The inhibition of DOPA synthesis, which was observed in the terminal field regions, was paralleled by decreases in the midbrain DA cell body regions. Ro 16-6028 potently inhibited *in vivo* TH activity

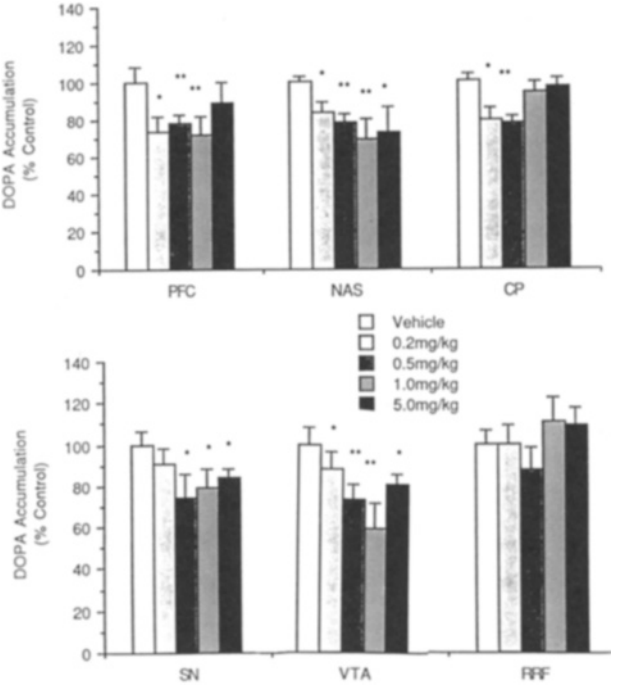

Fig. 1. Inhibitory effect of Ro 16-6028 on *in vivo* TH activity.

All animals received NSD-1015 (100 $mg \cdot kg^{-1}$) 5 min before various doses of Ro 16-6028. Each value of DOPA accumulation and standard error was shown in table 1.

$^{*}P < 0.05$, $^{**}P < 0.01$, relative to control value.

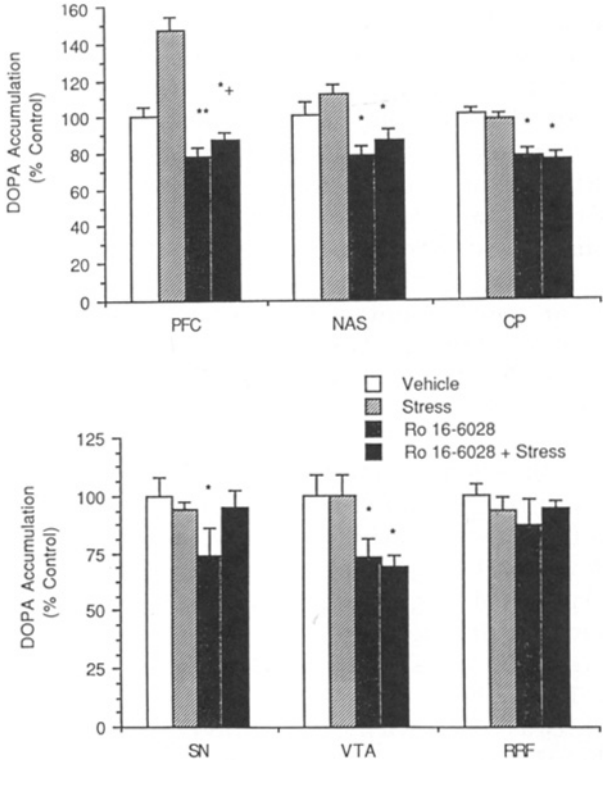

Fig. 2. Effect of Ro 16-6028 on *in vivo* TH activity with and without exposure to stress.

NSD-1015 (100 $mg \cdot kg^{-1}$) was injected 5 min after Ro 16-6028 (0.5 $mg \cdot kg^{-1}$), then animals were exposed to stress in some groups. Control value for each region is as follows: PFC; 1.78 ± 0.09, NAS; 19.71 ± 1.35, CP; 30.59 ± 0.92, SN; 9.04 ± 0.70, VTA; 28.16 ± 2.48 and RRF; 5.38 ± 0.27 ($ng \cdot mg^{-1}$ protein). Data were obtained from 5–6 animals per group.

$^{*}P < 0.05$, $^{**}P < 0.01$, relative to vehicle control.

$+P < 0.05$, relative to stress alone.

in VTA; source of the mesocortical and mesolimbic DA innervations, less potently inhibited the activity in SN; source of the striatal projection, and

did not inhibit in RRF; source of the striatal and limbic projection[8]. In contrast to the terminal field regions in which maximal inhibition of TH activity was obtained at the lowest dose tested (0.2 $mg \cdot kg^{-1}$), the cell body regions were less sensitive to the BDZ and the maximal inhibition was seen at 1.0 $mg \cdot kg^{-1}$ in VTA and SN.

Restraint stress remarkably increased *in vivo* TH activity (146.6%) in PFC (fig. 2). Pretreatment with Ro 16-6028 (0.5 $mg \cdot kg^{-1}$) prevented the stress-induced augmentation of TH activity in PFC and DOPA accumulation level following exposure to stress was still lower than control value. Thirty min restraint stress did not affect *in vivo* TH activity in NAS, CP and DA cell body regions tested.

Discussion

BDZ derivatives are commonly used in the clinical field, but they might conduct undesirable side effects due to their various pharmacological properties. The partial BDZ agonist Ro 16-6028 differs from other BDZs by virtue of absence of ethanol potentiation, minimal or no withdrawal symptomatology upon challenge with the BDZ antagonist flumazenil, and lack of motor impairment and muscle relaxation at doses employed in the present study[5]. Both BDZ and non-BDZ anxiolytic agents have been shown to reverse stress-induced augmentation of prefrontal cortical DA metabolism[1-3]. The present data indicate that Ro 16-6028 inhibits *in vivo* TH activity in mesotelencephalic DA system terminal field regions in a dose-related manner. Furthermore, Ro 16-6028 reverses the stress-elicited augmentation of TH activity in PFC. These findings are consistent with an anxiolytic profile. However, it is not clear to what degree other properties of BDZ agonists, especially sedation and muscle relaxation, contribute to the observed ability of these agents to reverse the stress-induced increases in prefrontal cortical DA metabolism. The present study indicates that muscle relaxant properties *per se* are not necessary to reverse the stress-induced augmentation of cortical DA metabolism, since muscle relaxation did not begin to appear in rats following Ro 16-6028 administration until doses of approximately 300 $mg \cdot kg^{-1}$[5].

It remains unclear to what degree the partial BDZ agonist nature of Ro 16-6028 contributes to the differential actions observed across the mesotelencephalic DA system. As noted before, the partial agonist is less potent in the DA cell body regions than in the terminal field regions. BDZ receptors are distributed to both the forebrain and midbrain regions examined. Recent data indicate that the anxiogenic β-carboline FG-7142 (an inverse agonist of BDZ receptors) increases *in vivo* TH activity in both the PFC and VTA, and that the β-carboline effects changes in DA synthesis through actions at both the terminal field and cell body levels, i.e., through both impulse-independent (presynaptic) and impulse-dependent means[9]. Ro 16-6028 may similarly act through both impulse-independent (terminal field level) and impulse-dependent (cell body level) actions. However, the lower potency of the BDZ at the cell body regions (VTA, SN and RRF) may suggest that the primary mode of action occurs at the terminal field level. It is also noteworthy that the β-carboline FG-7142 increases TH activity in PFC but decreases it in CP, i.e., exerts opposite actions in these two DA terminal fields[9]. In contrast, Ro 16-6028 uniformly depressed *in vivo* TH activity in the mesotelencephalic regions examined. The mechanism of anxiolytic action seems to be complicated and involved to multiple action sites.

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