Effects of μ-, δ-, and κ-Opioid Agonists and Enkephalinase Inhibitor RB101 in Two Inbred Rat Strains

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Analgesic and suppressive effects of selective μ- (DAGO), δ- (DME), and κ- (DAKLI) opioid agonists are compared with those of aminopeptidase N and neutral endopeptidase RB101 in rats of WAG/G and Fischer-344 rats. Fischer-344 rats were more susceptible to suppressive effects of DAGO and analgesic effect of DME. It is concluded that in these rats peculiarities of the μ- and δ-opioid systems determine susceptibility to locomotor depression and analgesia, respectively. There is no correlation between effects of DAGO and RB101 in these strains. This implies that depressive effect of RB101 is not mediated though μ-opioid systems. In contrast, the effects of DMA on pain sensitivity in WAG/G and F-344 rats are opposite to those of RB101. This suggests that specific features in the activity of cerebral δ-OS can determine the sensitivity of RB101-induced analgesia.

Key Words: *μ-, δ-, and κ-opioids; enkephalinase inhibitor; analgesia; motor activity*

Stimulation of endogenous opioid system (OS) produces various effects, in particular analgesia and locomotor depression [11,12]. Individual differences in the μ-, δ-, and κ-OS play an important role in realization of these effects [8]. For instance, reduced number of μ- and δ-opioid receptors in mouse brain is associated with decreased sensitivity to morphine-induced analgesia [7].

We have previously demonstrated that WAG/G and Fischer-344 (F-344) inbred rats considerably differ in morphine sensitivity. WAG/G rats are less susceptible to analgesic and suppressive actions and positive reinforcement with morphine, but less predisposed to the development of morphine dependence [10]. The number of binding sites for radiolabeled naloxone in WAG/G rats is increased [1].

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Prof. B. Roques and coworkers [3,9] demonstrated analgesic effects of RB101, a new potent inhibitor of aminopeptidase N and neutral endopeptidase (active upon systemic administration). Due to inhibition of all enzymes catalyzing enkephalin degradation RB101 considerably increases the concentration of endogenous enkephalins. Thus, the effects of RB101 are related primarily to cerebral δ-OS [2]. The possibility of using RB101 in clinical practice as an analgesic preparation and for treating drug addiction is now extensively studied in France.

To evaluate the role of individual genetic differences in μ-, δ-, and κ-OS we compared the effect of selective μ-, δ-, and κ-agonists with that of RB101 in two inbred rat strains (WAG/G and F-344).

MATERIALS AND METHODS

Experiments were performed on male WAG/G and F-344 rats weighing 180-200 g (64 rats of each strain). The animals were housed eight per cage and main-

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tained at 21°C. Test substances were injected though stainless steel cannulas implanted into the lateral brain ventricles under Nembutal anasthesia.

The following substances were used:

- N-{(R,S)-2-benzyl-3[(S)(2 amino-4-methylthio) butyl dithio]-1-oxopropyl}-1-phenylalanine benzyl ester (RB101), which crosses the blood-brain barrier and after reduction of disulfide bond the inactive molecule becomes a potent inhibitor of aminopeptidase N and neutral endopeptidase, the main enkephalin-metabolizing enzymes. Solvent for RB101 consisted of 10% ethanol, 10% cremophore EL, and 80% distilled water.
- [D-ALA^2,N-Me-Phe^4,Gly^5-ol]-enkephalin, selective agonist of μ-opioid receptors [5]
- Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2 (Dermenkephalin, DME), selective agonist of δ-opioid receptor [6].
- Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Arg-Leu-Arg-Gly 5 aminopeptylamide (DAKLI), selective agonists of κ-opioid receptors [4].

First, the baseline motor activity and pain sensitivity were assessed. To this end the rats were placed to an open field (40×50 cm) for 3 min, and vertical and horizontal activities were automatically assessed by counting crossings of square boundaries (2.5×2.5 cm) and 15-cm-height level, respectively. Immediately after the open field test the rats were placed into Plexiglass cylinders, and pain sensitivity was evaluated 4 times at 15-min intervals by the latency of tail withdrawal from hot (56°C) water. One week later the animals of each strain were divided into 8 groups. Group 1 rats received intraventricular injection of 5 μl isotonic NaCl, and group 2 rats were injected with 1 ml/kg RB101 solvent (in the caudal vein). Two groups of each strain were intravenously injected with 20 and 40 mg/kg RB101, respectively, and three groups of each strain received intraventricular injections of 1 mM DAGO, DME, or DAKLI. Motor activity and pain sensitivity were determined 5 min postinjection and presented as percentage of locomotor depression and analgesia. The data were processed statistically using the Student *t* test.

RESULTS

Parameters of baseline motor activity were similar in WAG/G and F-344 rats. RB101 solvent and NaCl had no effect on motor activity. RB101 induced a dose-dependent locomotor depression in both rat strains (Fig. 1). DAGO and DAKLI considerably suppressed motor activity. Effect of DAGO was more pronounced in F-344 rats, while DAKLI produced the same suppressive effect in both rat strains (Fig. 1). DMA had no effect on motor activity in both strains.

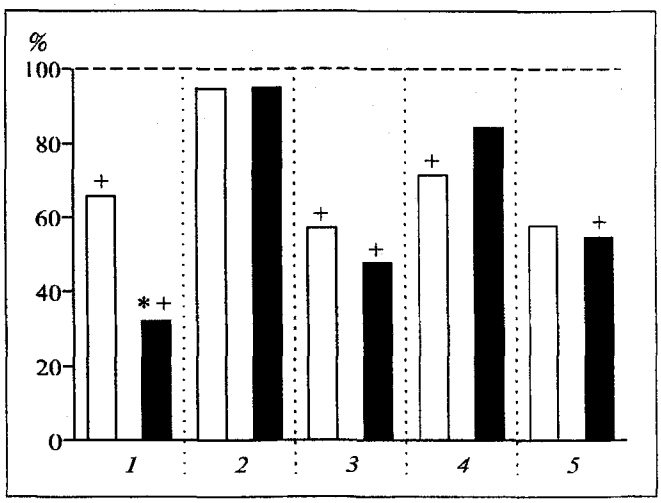

Fig. 1. Suppression of motor activity in WAG/G (open bars) and Fischer-344 (shaded bars) rats after injection of DAGO (*1*), DMA (*2*), DAKLI (*3*) and RB101 in doses 20 (*4*) and 40 mg/kg (*5*). Initial motor activity was taken as 100%. +p<0.05 compared with the initial level (dotted line). Here and in Figs. 2-4: *p<0.05 denote interstrain differences.

We have previously demonstrated that WAG/G rats are characterized by higher pain sensitivity [10]. As seen from Fig. 2, neither NaCl, nor RB101 solvent changed pain sensitivity in both rat strains. Injection of both doses of RB101 produced a considerable analgesic effect in WAG/G but not in F-344 rats (Fig. 3). DAGO produced minor effect in both strains (Fig. 4). Analgesic effect of DME was more pronounced in F-344 rats (p<0.01, Fig. 4). Administration of DAKLI produced slow analgesia in WAG/G rats and rapidly but transiently prolonged the latency of tail withdrawal in F-344 rats (Fig. 4).

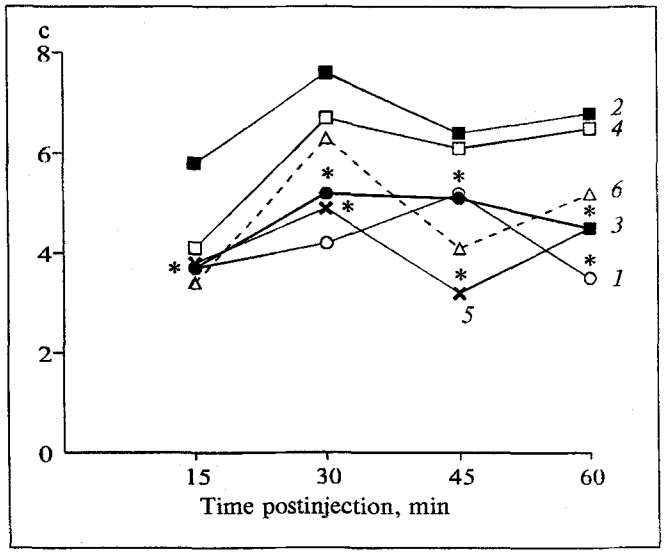

Fig. 2. Latency of tail withdrawal in WAG/G (*1*, *3*, *5*) and Fischer-344 (*2*, *4*, *6*) rats. *1*, *2*) initial level; 3, 4) intraventricular injection of NaCl; *5*, *6*) injection of RB101 solvent into caudal vein.

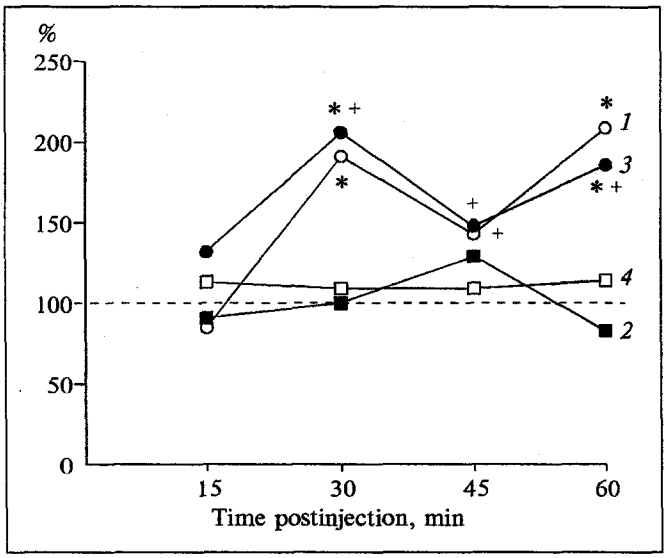

Fig. 3. Changes (%) in latency of tail withdrawal in WAG/G (*1*, *3*) and Fischer-344 (*2*, *4*) rats after injection of 20 (*1*, *2*) and 40 mg/kg (*3*, *4*) RB101. Here and in Fig 4: +$p<0.05$ compared with the control (100%).

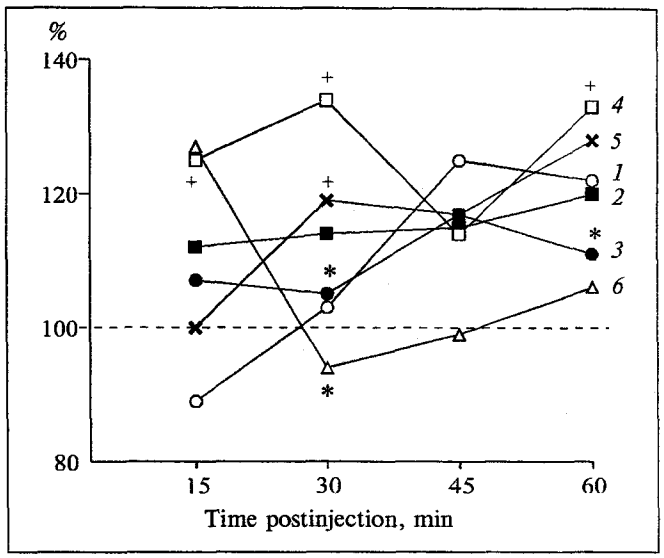

Fig. 4. Changes (%) in latency of tail withdrawal in WAG/G (*1*, *3*, *5*) and Fischer-344 (*2*, *4*, *6*) rats after injection of DAGO (*1*, *2*) and DME (*3*, *4*) and DAKLI (*5*, *6*).

Thus, DAKLI induced opposite changes in pain sensitivity in F-344 and WAG/G rats ($p<0.01$).

Our experiment revealed substantial differences in the suppressive effect of DAGO and analgesic effect of DME between WAG/G and F-344 rats. In both cases F-344 rats were characterized by higher sensitivity. It can be concluded that some peculiarities of in μ- and δ-OS in F-344 rats facilitate the development of locomotor depression and analgesia, respectively. Effect of DAGO in these strains did not correlate with that of RB101. This implies that peculiarities in the cerebral μ-OS are not essential for the formation of RB101-induced locomotor depression. On the contrary, effects of DMA on pain sensitivity in WAG/G and F-344 rats were opposite to those of RB101. This suggest that individual peculiarities in activity of the cerebral δ-OS can determine the sensitivity of RB101-induced analgesia. Individual sensitivity to the analgesic and suppressive effects of RB101 are apparently independent on the cerebral κ-OS.

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