Differential Effects of Buprenorphine and Pentazocine on the Regional Cerebral Metabolic Rate for Glucose in the Conscious Rat

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The effects of buprenorphine (BNP, 10–200 $\mu\text{g/kg}$, i.v.) and pentazocine (PTZ, 2.5–10 mg/kg , i.v.) on the regional cerebral metabolic rate for glucose ($r\text{CMR}_{\text{glc}}$) were analyzed in nine anatomically discrete areas of the conscious rat brain by the simultaneous use of [^{14}C]2-deoxyglucose, the glucose analogue that can be phosphorylated in the brain, and [^3H]3-*O*-methylglucose, a nonmetabolizable glucose analogue. Originally, this method was developed by Gjedde and Diemer in the rat and in humans. The $r\text{CMR}_{\text{glc}}$ was significantly decreased by BNP (100 or 200 $\mu\text{g/kg}$) in most of the brain regions investigated, except the cerebellum. In contrast, PTZ (10 mg/kg) significantly increased $r\text{CMR}_{\text{glc}}$ in the cerebral cortex and medulla. In the cerebral cortex and medulla, the direction of the effect on $r\text{CMR}_{\text{glc}}$ was opposite for BNP (22% decrease at the dose of 200 $\mu\text{g/kg}$) and PTZ (22% increase at the dose of 10 mg/kg). These findings strongly suggest that the discrepancies between the marked effects of BNP (a partial μ agonist and κ antagonist) and PTZ (a μ antagonist and κ agonist) on $r\text{CMR}_{\text{glc}}$ reflect the selectivity of agonist action at the different types of opioid receptors, μ and κ receptors, in the rat brain.

Key words regional cerebral metabolic rate for glucose; buprenorphine; pentazocine; conscious rat

Progress in understanding the mechanisms of actions of the opioids and in developing opioid analgesics that are less likely to result in opioid-dependence has been derived from the discovery of the opioid mixed agonist/antagonists. Mixed agonist/antagonists may act as agonists or antagonists depending upon the behavioral pharmacological study and the degree of receptor activation that is required to elicit agonistic effects.¹⁾ Buprenorphine (BNP) and pentazocine (PTZ), among several opioid mixed agonist/antagonists, have been characterized on the basis of intrinsic pharmacological activity^{2,3)} and classified according to receptor activity: BNP-partial μ agonist, κ antagonist^{4–8)}; PTZ- μ antagonist, κ agonist.^{7,8)}

The concept of multiple opioid receptors in the central nervous system is generally accepted^{2,7)} and is consistent with observed differences in physiological and behavioral responses to various exogenous opioids.⁸⁾ Diverse distribution of the opioid receptors in rat brain has been extensively investigated using *in vivo* autoradiography^{9–12)} and filtration binding assays on brain homogenate *in vitro*.^{13–15)} Nonetheless, it has not been established that the brain areas which show significant densities of the receptors demonstrate physiological responses to the mixed agonist/antagonists. Furthermore, the potential for functional *in vivo* effects of the mixed agonist/antagonists propagated to brain regions remote from initial interactions with the receptors has not been explored.

The [^{14}C]2-deoxyglucose ([^{14}C]2-DG) technique is a tool which has been developed to trace alterations of brain functional activity by measurement of the regional cerebral metabolic rate for glucose ($r\text{CMR}_{\text{glc}}$) because, like oxygen, glucose is a major substrate for cerebral energy metabolism.^{16–18)} In the present study, using the [^{14}C]2-DG technique, we have measured $r\text{CMR}_{\text{glc}}$ in several regions of rat brain following the intravenous (i.v.) administration of BNP or PTZ, in an attempt to identify specific areas in which functional events could be altered by the mixed agonist/antagonists. A preliminary report of this work has appeared as an abstract.¹⁹⁾

MATERIALS AND METHODS

Experimental Animals Female Wistar rats weighing 180–200 g (9 weeks of age), were obtained from Japan SLC, Inc. (Shizuoka, Japan). The rats were housed at $23 \pm 1^\circ\text{C}$ and a humidity of $55 \pm 10\%$ under a 12 h light/dark cycle, and had free access to food and water.

Chemicals The drugs used in the present study were BNP (Lepetan injection, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and PTZ (Sosegon injection, Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan). The BNP and PTZ solutions were prepared at concentrations of 10, 100 and 200 $\mu\text{g/ml}$, and 2.5, 5 and 10 mg/ml in physiological saline (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), respectively. [^3H]3-*O*-methylglucose ([^3H]3-*O*-MG, specific activity 2782.4 GBq (75.2 Ci)/mmol) and [^{14}C]2-DG (specific activity 2.1 GBq (57.2 mCi)/mmol), were purchased from New England Nuclear Co. (Boston, MA, U.S.A.). Soluene-350, Pico-Fluor 40 and Hionic-Fluor were purchased from Packard Instruments Co., Inc. (Downers Grove, IL, U.S.A.). All other chemicals were of at least guaranteed reagent grade.

Surgical Operation Under light halothane (Fluothane, Takeda Chemical Industries, Ltd., Osaka, Japan) anesthesia, the left femoral artery and the right femoral vein were catheterized with polyethylene tubing (PE 50, i.d., 0.58 mm; o.d., 0.965 mm; Clay Adams, Parsippany, NJ, U.S.A.) and, after surgery, animals were partially restrained by a loose-fitting abdominal-pelvic plastic cast and taped to lead bricks for support. There were no apparent indications of stress in this partially restrained condition. The animals were left undisturbed under quiet circumstances to recover from the anesthesia for at least 1 h after the end of surgical operation and were then used for the experiments. All procedures were finished between 9:00 a.m. and 12:00 noon to minimize the day's variations, because the existence of a circadian rhythm in $r\text{CMR}_{\text{glc}}$ has been reported by Crane *et al.*²⁰⁾

Measurement of Physiological Parameters Rectal tem-

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perature was monitored continuously with a precision digital thermometer (DP-200C, Rika Kogyo Co., Ltd., Tokyo, Japan) and maintained at 35–37°C using heat lamps, because it has been reported that $rCMR_{\text{glc}}$ was decreased in hypothermic rats²¹⁾ and also during anesthesia in the rat^{16,22)} and mouse.²³⁾ The hematocrit and the arterial pH were measured just before each experiment. Simultaneously, the glucose concentration in the arterial plasma (C_a) was measured by a commercial kit (Glucose CII-test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Measurement of $rCMR_{\text{glc}}$ $rCMR_{\text{glc}}$ was measured by a modification of the method of Gjedde and Diemer.²⁴⁾ Briefly, just after i.v. administration of BNP (10–200 µg/kg) or PTZ (2.5–10 mg/kg) over a 10-s period, a 100 µl mixture of 185 kBq (5 µCi) [³H]3-*O*-MG and 37 kBq (1 µCi) [¹⁴C]2-DG in saline was injected into the femoral vein and circulated for 10 min. Control animals were treated with physiological saline solution (1 ml/kg). Arterial blood samples were taken at 1, 2, 3, 4, 6, 8 and 10 min for the determination of the [³H]3-*O*-MG and [¹⁴C]2-DG plasma concentration integrals. Blood samples were immediately centrifuged at 2000 × *g* for 5 min at 4°C. Trunk blood was obtained to measure the plasma glucose concentration by the Glucose CII-test Wako. After 10 min of tracer circulation, animals were sacrificed by decapitation. Brains were rapidly dissected and divided into nine parts: the frontal cortex, parietal cortex, temporal cortex, occipital cortex, medulla, midbrain, thalamus, cerebellum and medulla oblongata including the pons. Each tissue was weighed and solubilized in 1 ml of Soluene-350 at 50°C for 2–4 h, then 10 ml of Hionic-Fluor, the scintillation cocktail, was added to each vial. Plasma samples in a volume of 100 µl were dissolved in 3 ml of Pico-Fluor 40. The [³H]3-*O*-MG and [¹⁴C]2-DG contents in tissue and plasma samples were determined by a liquid scintillation counter (TRI-CARB 2050CA, Packard Instruments Co., Inc., Downers Grove, IL, U.S.A.).

Calculations The phosphorylation clearance of [¹⁴C]2-DG (K_D) and lumped constant (LC) were calculated according to the following equations (Eqs. 1 and 2).²⁵⁾

$$K_D = [M_{\text{total}}^*(T) - V_M \times C_a^*(T)] / \int_0^T C_a^*(t) dt \quad (1)$$

where T is the decapitation time, $M_{\text{total}}^*(T)$ is the total tissue radioactivity derived from labeled [¹⁴C]2-DG, V_M

is the distribution volume of [³H]3-*O*-MG and $C_a^*(T)$ is the radioactive [¹⁴C]2-DG concentration in arterial plasma.

$$LC = (k_3^*/k_3) \times (V_M \times C_a^G) / [V_M \times (K_i + C_a^G) - K_e] \quad (2)$$

where k_3^* and k_3 are the rate constants for the phosphorylation of [¹⁴C]2-DG and glucose, respectively, C_a^G is the plasma glucose concentration in the trunk blood, K_i is the Michaelis constant of glucose transport from blood to brain ($K_i = 3.7$ mM),²⁵⁾ $K_e (= K_i \times V_d)$ is the half saturation constant of brain glucose transport from brain to blood, and V_d is the water volume of the brain, the actual volume of distribution of glucose ($V_d = 0.8$ ml/g brain),²³⁾ respectively. We used a universal value of 0.3 for the phosphorylation ratio (k_3^*/k_3).²⁴⁾ $rCMR_{\text{glc}}$ was calculated as follows:

$$rCMR_{\text{glc}} = C_a^G \times K_D / LC \quad (3)$$

Statistical Analysis Data are expressed as the mean ± S.E.M. Statistical analysis of results was performed by analysis of variance (ANOVA) followed by Dunnett's multiple range test for multiple comparisons. A p value of less than 0.05 was considered statistically significant.

RESULTS

Physiological Parameters The physiological parameters in the conscious rats are listed in Table 1. No significant difference between the groups of control and BNP or PTZ was noted in any of the physiological parameters (body weight, body temperature, hematocrit, arterial pH and C_a) measured just before each experiment. After the injection of the tracers, various doses of BNP (10–200 µg/kg) or PTZ (2.5–10 mg/kg) had no significant effects on C_a^G when compared to the control group.

Values of K_D and LC Tables 2 and 3 show the effects of BNP and PTZ administration on the phosphorylation clearance of [¹⁴C]2-DG (K_D) and the lumped constant (LC) in various brain regions in the conscious rats, compared with those in the control. The values of K_D and LC based on the double tracer technique can be calculated according to Eqs. 1 and 2, respectively. The regional average of K_D varied from 0.024 (cerebellum) to 0.035 (frontal cortex) ml/min/g brain in the control rats (Table 2). The average value of the K_D in whole brain was 0.030 ml/min/g brain. This value was close to the average

Table 1. Physiological Parameters in the Conscious Rats

Dose	Body weight (g)	Body temperature (°C)	Hematocrit (%)	Arterial pH	$C_a^a)$ (mM)	$C_a^{Gb})$ (mM)
Control	198 ± 4	36.3 ± 0.3	49.3 ± 1.5	7.40 ± 0.04	11.6 ± 0.9	15.3 ± 0.9
BNP (µg/kg)						
10	206 ± 1	36.0 ± 0.2	47.6 ± 1.3	7.33 ± 0.03	10.7 ± 0.3	13.6 ± 0.6
100	204 ± 3	36.8 ± 0.3	48.0 ± 0.6	7.35 ± 0.03	11.9 ± 0.4	14.0 ± 1.1
200	205 ± 4	35.8 ± 0.4	47.1 ± 0.8	7.43 ± 0.05	11.4 ± 0.9	15.5 ± 0.9
PTZ (mg/kg)						
2.5	201 ± 2	36.0 ± 0.4	50.3 ± 0.4	7.38 ± 0.05	12.0 ± 0.7	16.9 ± 1.0
5	201 ± 2	36.0 ± 0.4	50.3 ± 0.8	7.43 ± 0.03	11.7 ± 0.5	16.2 ± 0.6
10	196 ± 2	35.9 ± 0.1	50.0 ± 0.4	7.38 ± 0.05	12.2 ± 0.3	16.6 ± 0.4

a) The glucose concentration in the arterial plasma just before the experiment. b) The plasma glucose concentration in the trunk blood after the experiment. Values represent the mean ± S.E.M. of four independent experiments. No significant difference from control (Dunnett's test, $p < 0.05$).

Table 2. Effects of BNP and PTZ Administration on Phosphorylation Clearance of [^{14}C]2-DG (K_D)

Region	Phosphorylation clearance of [^{14}C]2-DG (K_D) (ml/min/g brain)						
	Control	BNP ($\mu\text{g/kg}$)			PTZ (mg/kg)		
		10	100	200	2.5	5	10
Frontal cortex	0.035 $\pm$ 0.003	0.038 $\pm$ 0.002	0.028 $\pm$ 0.002	0.025 $\pm$ 0.001*	0.031 $\pm$ 0.002	0.035 $\pm$ 0.002	0.038 $\pm$ 0.001
Parietal cortex	0.030 $\pm$ 0.002	0.033 $\pm$ 0.002	0.026 $\pm$ 0.001	0.023 $\pm$ 0.002	0.029 $\pm$ 0.002	0.032 $\pm$ 0.001	0.039 $\pm$ 0.001*
Temporal cortex	0.032 $\pm$ 0.002	0.035 $\pm$ 0.002	0.029 $\pm$ 0.002	0.024 $\pm$ 0.001*	0.031 $\pm$ 0.002	0.032 $\pm$ 0.002	0.036 $\pm$ 0.001
Occipital cortex	0.032 $\pm$ 0.003	0.037 $\pm$ 0.003	0.029 $\pm$ 0.002	0.026 $\pm$ 0.002	0.030 $\pm$ 0.003	0.032 $\pm$ 0.001	0.035 $\pm$ 0.001
Medulla	0.026 $\pm$ 0.001	0.029 $\pm$ 0.001	0.024 $\pm$ 0.001	0.021 $\pm$ 0.001*	0.023 $\pm$ 0.001	0.026 $\pm$ 0.001	0.028 $\pm$ 0.001
Midbrain	0.031 $\pm$ 0.002	0.033 $\pm$ 0.002	0.028 $\pm$ 0.002	0.024 $\pm$ 0.002	0.029 $\pm$ 0.003	0.028 $\pm$ 0.001	0.028 $\pm$ 0.001
Thalamus	0.030 $\pm$ 0.002	0.029 $\pm$ 0.002	0.024 $\pm$ 0.002	0.021 $\pm$ 0.001*	0.025 $\pm$ 0.001	0.026 $\pm$ 0.001	0.026 $\pm$ 0.001
Cerebellum	0.024 $\pm$ 0.001	0.029 $\pm$ 0.001	0.023 $\pm$ 0.001	0.022 $\pm$ 0.001	0.023 $\pm$ 0.002	0.023 $\pm$ 0.001	0.023 $\pm$ 0.001
Medulla oblongata	0.026 $\pm$ 0.002	0.029 $\pm$ 0.002	0.025 $\pm$ 0.001	0.021 $\pm$ 0.002	0.024 $\pm$ 0.002	0.024 $\pm$ 0.001	0.024 $\pm$ 0.001

Values represent the mean $\pm$ S.E.M. of four independent experiments. For the calculation of the K_D , see Eq. 1 in "Materials and Methods". * $p < 0.05$, significant difference from control (Dunnett's test).

Table 3. Effects of BNP and PTZ Administration on Lumped Constant (LC)

Region	Lumped constant (LC)						
	Control	BNP ($\mu\text{g/kg}$)			PTZ (mg/kg)		
		10	100	200	2.5	5	10
Frontal cortex	0.46 $\pm$ 0.01	0.47 $\pm$ 0.02	0.43 $\pm$ 0.01	0.45 $\pm$ 0.01	0.46 $\pm$ 0.01	0.45 $\pm$ 0.01	0.45 $\pm$ 0.01
Parietal cortex	0.45 $\pm$ 0.01	0.45 $\pm$ 0.01	0.45 $\pm$ 0.02	0.45 $\pm$ 0.01	0.45 $\pm$ 0.01	0.44 $\pm$ 0.01	0.50 $\pm$ 0.03
Temporal cortex	0.45 $\pm$ 0.01	0.44 $\pm$ 0.01	0.44 $\pm$ 0.01	0.44 $\pm$ 0.01	0.47 $\pm$ 0.01	0.45 $\pm$ 0.01	0.46 $\pm$ 0.01
Occipital cortex	0.46 $\pm$ 0.01	0.49 $\pm$ 0.02	0.44 $\pm$ 0.01	0.47 $\pm$ 0.03	0.46 $\pm$ 0.01	0.45 $\pm$ 0.01	0.48 $\pm$ 0.01
Medulla	0.50 $\pm$ 0.02	0.48 $\pm$ 0.01	0.47 $\pm$ 0.01	0.51 $\pm$ 0.02	0.46 $\pm$ 0.01	0.48 $\pm$ 0.01	0.49 $\pm$ 0.01
Midbrain	0.47 $\pm$ 0.01	0.45 $\pm$ 0.01	0.46 $\pm$ 0.01	0.46 $\pm$ 0.01	0.50 $\pm$ 0.02	0.45 $\pm$ 0.01	0.47 $\pm$ 0.01
Thalamus	0.47 $\pm$ 0.02	0.45 $\pm$ 0.01	0.44 $\pm$ 0.01	0.45 $\pm$ 0.01	0.45 $\pm$ 0.01	0.45 $\pm$ 0.01	0.46 $\pm$ 0.01
Cerebellum	0.43 $\pm$ 0.01	0.45 $\pm$ 0.01	0.43 $\pm$ 0.01	0.46 $\pm$ 0.03	0.44 $\pm$ 0.01	0.43 $\pm$ 0.01	0.45 $\pm$ 0.01
Medulla oblongata	0.51 $\pm$ 0.01	0.52 $\pm$ 0.03	0.55 $\pm$ 0.02	0.51 $\pm$ 0.01	0.52 $\pm$ 0.01	0.50 $\pm$ 0.01	0.51 $\pm$ 0.01

Values represent the mean $\pm$ S.E.M. of four independent experiments. For the calculation of the LC , see Eq. 2 in "Materials and Methods". No significant difference from control (Dunnett's test, $p < 0.05$).

Table 4. Effects of BNP and PTZ on Regional Cerebral Metabolic Rates for Glucose ($rCMR_{\text{glc}}$) in the Rat Brain at 10 min after i.v. Administration of BNP or PTZ

Region	Regional cerebral metabolic rates for glucose ($rCMR_{\text{glc}}$) ($\mu\text{mol/min/100 g brain}$)						
	Control	BNP ($\mu\text{g/kg}$)			PTZ (mg/kg)		
		10	100	200	2.5	5	10
Frontal cortex	114 $\pm$ 2	109 $\pm$ 4	90 $\pm$ 2*	85 $\pm$ 1*	114 $\pm$ 5	127 $\pm$ 2*	141 $\pm$ 2*
Parietal cortex	102 $\pm$ 5	97 $\pm$ 2	80 $\pm$ 4*	78 $\pm$ 2*	109 $\pm$ 4	117 $\pm$ 2	131 $\pm$ 4*
Temporal cortex	107 $\pm$ 3	109 $\pm$ 3	91 $\pm$ 2*	85 $\pm$ 2*	110 $\pm$ 4	112 $\pm$ 2	132 $\pm$ 2*
Occipital cortex	105 $\pm$ 4	104 $\pm$ 5	88 $\pm$ 4*	85 $\pm$ 4	109 $\pm$ 2	116 $\pm$ 1	122 $\pm$ 5*
Medulla	79 $\pm$ 3	82 $\pm$ 2	70 $\pm$ 2	63 $\pm$ 2*	84 $\pm$ 1	89 $\pm$ 2	94 $\pm$ 3*
Midbrain	100 $\pm$ 1	99 $\pm$ 3	83 $\pm$ 3*	80 $\pm$ 4*	98 $\pm$ 4	100 $\pm$ 3	98 $\pm$ 4
Thalamus	96 $\pm$ 4	86 $\pm$ 2	74 $\pm$ 3*	73 $\pm$ 3*	92 $\pm$ 2	93 $\pm$ 4	96 $\pm$ 4
Cerebellum	84 $\pm$ 2	86 $\pm$ 3	74 $\pm$ 3	73 $\pm$ 3	87 $\pm$ 2	87 $\pm$ 1	87 $\pm$ 5
Medulla oblongata	78 $\pm$ 1	74 $\pm$ 3	63 $\pm$ 4	63 $\pm$ 4*	77 $\pm$ 2	79 $\pm$ 3	77 $\pm$ 5

Values represent the mean $\pm$ S.E.M. of four independent experiments. For the calculation of the $rCMR_{\text{glc}}$, see Eq. 3 in "Materials and Methods". * $p < 0.05$, significant difference from control (Dunnett's test).

of an earlier K_D (0.028 ml/min/g brain) in male Wistar rats reported by Gjedde.²⁶ The K_D of BNP were significantly decreased in the presence of the highest dose (200 $\mu\text{g/kg}$) of BNP in the frontal cortex, temporal cortex, medulla and thalamus compared to the control group, whereas the value of K_D was significantly increased by PTZ (10 mg/kg)

treatment in the parietal cortex. In every region of the brain, the lower dose of BNP or PTZ statistically left the K_D values unchanged compared to the control group. On the other hand, each administration group of BNP (10–200 $\mu\text{g/kg}$) or PTZ (2.5–10 mg/kg) had no significant effect on the LC value compared to the control group in

every region of brain (Table 3).

Regional Cerebral Metabolic Rate for Glucose ($rCMR_{glc}$)
In Table 4, changes in the $rCMR_{glc}$ in the rat brain regions at 10 min after i.v. administration of BNP or PTZ are listed. The values of $rCMR_{glc}$ were calculated by Eq. 3 using the values of C_a^G listed in Table 1 and the values of LC and K_D listed in Tables 2 and 3. The $rCMR_{glc}$ in the control rats in this study was similar to that previously reported for intact conscious rats.^{17,27,28} The administration of BNP significantly reduced $rCMR_{glc}$ in the frontal cortex (21 and 25% at doses of 100 and 200 $\mu\text{g/kg}$, respectively), parietal cortex (22 and 24%), temporal cortex (15 and 21%), midbrain (17 and 20%), thalamus (23 and 24%), occipital cortex (16% at the dose of 100 $\mu\text{g/kg}$) and medulla (20% at the dose of 200 $\mu\text{g/kg}$). The magnitude of the dose-dependent reduction in $rCMR_{glc}$ response to BNP was relatively uniform among the measured regions. No statistically significant effect of BNP was demonstrated at the dose of 10 $\mu\text{g/kg}$ of BNP. A significant increase in $rCMR_{glc}$ by PTZ was localized to the frontal cortex (11 and 24% at the dose of 5 and 10 mg/kg, respectively), parietal cortex (28% at the dose of 10 mg/kg), temporal cortex (23%), occipital cortex (16%) and medulla (19%). At the lowest dose of PTZ (2.5 mg/kg), no significant difference was observed in the $rCMR_{glc}$ value in any region of the brain.

DISCUSSION

The original [^{14}C]2-DG autoradiographic method of Sokoloff *et al.*¹⁶ has been of great utility in characterizing functional cerebral anatomy. The physiological activity of neurons requires energy derived from the metabolism of glucose. By mapping regional cerebral glucose utilization, inferences can be made about regional physiological neuronal activity. Several investigators have tried to map cerebral metabolic responses to various opioids using this method.^{29–39} In these previous studies, the estimation of regional glucose phosphorylation is based on regional [^{14}C]2-DG accumulation. However, the regional, pathological or pharmacological variability of the isotope correction factor, the so-called “lumped constant” (LC), between [^{14}C]2-DG accumulation and glucose consumption in the brain was not considered in the method of Sokoloff *et al.*¹⁶ The LC may change under the regional, pathophysiological and pharmacological conditions in which the relationship between the plasma and brain glucose level has changed. Indeed, Gjedde²⁶ has reported that LC increased when the balance between the supply and consumption of glucose was shifted towards a lower glucose content in the brain. Therefore, the magnitude of LC in the pathological and/or drug-treated tissue must be determined previously, in order to calculate glucose consumption from the [^{14}C]2-DG accumulation. Recently, the method of Gjedde and Diemer²⁴ has allowed the regional determination of LC in the brain by the simultaneous use of [^{14}C]2-DG and [^3H]3-*O*-MG (nonmetabolizable glucose analog). Furthermore, the use of both [^{14}C]2-DG and [^3H]3-*O*-MG allowed the determination of regional cerebral glucose utilization to be made within 10 min of the tracer administration, rather

than the usual 45 min^{24,25} required for the original [^{14}C]2-DG method. Therefore, this method makes it possible to determine a rapid alteration of brain functional activity at 10 min after the administration of the drugs.¹⁹ We adopted this technique in the present study because maximum analgesia has been observed 10 min after the i.v. administration of BNP (8 $\mu\text{g/kg}$)⁴⁰ or PTZ (2.5–10 mg/kg)⁴¹ to rats.

In the present study, the LC in the control rats varied insignificantly, between 0.43 and 0.51 in every brain region, with an overall average of 0.47, corresponding closely to the confirmed value (0.46) of Sokoloff¹⁷ in conscious rats (Table 2). In addition, the LC and the C_a^G (Table 1) in the drug-treated rats were not markedly changed compared to the control group, therefore, it is suggested that the observed alterations in $rCMR_{glc}$ are probably not caused by a secondary effect such as a hyperglycemic effect, variation of regional cerebral blood flow, heart rate, blood pressure or respiratory rate by the drugs.

The density and pattern of the distribution of opioid receptors have been widely characterized throughout the rat brain. Several studies have revealed a high concentration of μ and κ receptors in the thalamus, midbrain, cerebral cortex and medulla, whereas very low densities were present in the cerebellum.^{9–12} In the present study, the effects of BNP and PTZ on $rCMR_{glc}$ followed the distribution of opioid receptors in the rat brain to some extent. For example, the cerebellum did not exhibit any alteration of metabolism in response to BNP or PTZ, because of low to negligible levels of opioid receptors. However, in the brain regions containing high densities of μ and κ receptors, such as the thalamus and midbrain, the $rCMR_{glc}$ was unaffected by PTZ. From this point of view, we can predict that the distribution of amount of changes in $rCMR_{glc}$ in several brain regions would not directly parallel the density and pattern of the distributions of relevant opioid receptors in the brain. Discrepancies between $rCMR_{glc}$ changes and the distribution of relevant receptors in the brain have been observed using the [^{14}C]2-DG method in rats treated with a variety of drugs, including muscarinic agonists,^{42,43} and agonists and antagonists for γ -aminobutyric acid (GABA_A) receptors.⁴⁴

The $rCMR_{glc}$ was significantly decreased by BNP (100 or 200 $\mu\text{g/kg}$) in the cerebral cortex, medulla, midbrain, thalamus and medulla oblongata. In contrast, PTZ (10 mg/kg) significantly increased $rCMR_{glc}$ in the cerebral cortex and medulla. The overall effects of BNP and PTZ were consistent with previous findings demonstrating changes in $rCMR_{glc}$ in response to BNP in human (21% decrease)³⁸ and to (+)-pentazocine in rats (16% increase),³⁹ respectively. In the cerebral cortex and medulla, the direction of the effect on $rCMR_{glc}$ was opposite for BNP (22% decrease at the dose of 200 $\mu\text{g/kg}$) and PTZ (22% increase at the dose of 10 mg/kg). This opposite effect may be attributable to a difference in the potencies or receptor binding profiles of the drugs. Since the values of $rCMR_{glc}$ in general reflect local and direct interactions with the receptors as well as secondary changes that are due to metabolism in afferents, it is suggested that the changes in $rCMR_{glc}$ in response to BNP and PTZ prop-

agate to brain regions remote from the initial and direct interactions with the κ and μ binding sites of opioid receptors. Support for this assertion is derived from the comparison of selectivity of the agonist action of two types of mixed agonist/antagonists at different types of opioid receptors in the brain. BNP has partial μ agonist and κ antagonist properties, whereas PTZ is a μ antagonist and κ agonist. In previous studies, morphine,²⁹⁻³⁴ a full agonist acting primarily at μ receptors with a lower affinity for δ and κ receptors,⁸ as well as the other μ agonist (sufentanil)³⁶ decreased the $rCMR_{glc}$ in rats. Fanelli *et al.*³⁷ have reported that significant differences in the effects on $rCMR_{glc}$ were observed between a κ agonist (nalbuphine) and the two μ agonists, morphine and oxymorphone. In that report, the two μ agonists decreased the $rCMR_{glc}$, however, the κ agonist increased the $rCMR_{glc}$ in rat brain.³⁷ Consequently, it is strongly suggested that BNP, which has μ agonist properties, decreases $rCMR_{glc}$ in the brain, and PTZ, which has κ agonist properties, increases $rCMR_{glc}$. These findings lead us to presume that the double tracer technique may be useful in distinguishing between μ and κ agonist mechanisms.

The present study is the first demonstration regarding the differential effects of BNP and PTZ on $rCMR_{glc}$ which were measured by the double tracer technique with the same i.v. route and a 10-min period of drug administration. We can predict that this *in vivo* effect on $rCMR_{glc}$ correlates to the maximum analgesia in behavior at 10 min following the i.v. administration of BNP or PTZ to rats as previously reported.^{40,41} Moreover, the results reported here strongly suggest that the discrepancies between the findings in the two drugs may reflect the selectivity of agonist action at the different types of opioid receptors, μ and κ receptors, in the rat brain.

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