Pharmacokinetics of Pentazocine and Its Occupancy of Opioid Receptors in Rat Brain

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In order to assess quantitatively the pharmacodynamic process of pentazocine (PTZ), time courses of its plasma concentration and of the occupation of specific opioid receptors in the brain were investigated after intravenous (i.v.) administration of PTZ to rats. The plasma concentration of PTZ was determined by HPLC and the pharmacokinetic parameters were analyzed using nonlinear least-squares analysis. Measurement of ex vivo receptor occupation was made by comparing the specific [3H]naloxone (opioid receptor antagonist) binding in vitro to the crude P$_2$-synaptosomal fractions between vehicle-treated rats (control) and PTZ-treated rats. Following the i.v. administration of PTZ, the occupancy of specific opioid receptors decreased rapidly until 10 min, depending on the two pharmacological doses (2.5 and 10 mg/kg). The results strongly suggest the fast binding kinetics of PTZ in terms of its association with and dissociation from specific opioid receptor sites in the brain in addition to its fast rate of disappearance from the brain compartment. Furthermore, we demonstrated that the time profile of receptor occupancy correlated well (r=0.8650) with that of the unbound concentration in plasma until 120 min after the i.v. administration of PTZ to rats.

Key words  pentazocine; receptor occupancy; pharmacokinetics

Pentazocine (PTZ), a relatively short-acting analgesic, is useful for clinical application in the relief of cancer-related and postoperative pain because it is a reasonably strong analgesic with lower abuse liability than morphine.1) To characterize the disposition of PTZ in blood or different body tissues, pharmacokinetic studies of this drug at present have been extensively revealed in humans2,3) and in several animal species, including mouse,4) rat,5–7) rabbit,8) cat,9) dog,10) monkey.11,12) The time course of the analgesic effect of PTZ has also been investigated in various behavioral pharmacological models. After the intravenous (i.v.) administration of PTZ to rats, the analgesic effect emerges gradually during an early time period and its disappearance is rapid.13,14) Similar results were also obtained by Paalzow and Arbin in the mouse4) and Berkowitz et al. in human.15) Furthermore, we recently demonstrated that PTZ increased cerebral glucose utilization (the cerebral neuronal activity) within 10 min in the rat, and that this increase was correlated with the peak analgesia of PTZ (2.5—10 mg/kg, i.v.) at 10 min in a dose-dependent manner.13) These changing pharmacodynamic responses at an early time period must mainly involve PTZ interaction with specific opioid receptors in the central nervous system. However, the kinetic process of the brain receptor occupation by PTZ has not been followed up previously in vitro or under more physiological conditions (in vivo). Therefore, the object of this investigation is to characterize the time-dependent change of the specific receptor binding of PTZ in vivo in order to more quantitatively assess the pharmacodynamic responses of this drug.

The relationship between drug concentration in the blood and analgesic effect provides the basis for the clinical use of pharmacokinetics in improving drug therapy.16–18) Furthermore, it has recently been suggested that the receptor occupation by the drug is a more appropriate indicator of the pharmacological effect of the drug than its concentration in plasma or tissue, by Sawada et al.19)

Thus, the in vivo or ex vivo characterization of PTZ binding to the receptors appears to be more important for analysis of the pharmacokinetics and pharmacodynamics of this drug.

In the present study, using the ex vivo technique (in vitro measurement of radiolabeled ligand binding after in vivo treatment of the drug), we investigated the time profile of the brain receptor occupation after i.v. administration of PTZ to rats. We also discussed the relationship between its receptor occupation and plasma concentration.

MATERIALS AND METHODS

Experimental Animals  Female Wistar rats, aged 8 weeks (Saitama Experimental Animals Supply Co., Ltd., Saitama, Japan), were housed in stainless steel cages with a fixed light/dark cycle (8:00 a.m. to 8:00 p.m.) under conditions of controlled temperature maintained at 23 ± 1°C with a humidity of 55 ± 10% for at least 1 week before being used. Rats weighing 180—200 g were used throughout all the experiments. Food and water were made available continuously.

Chemicals  PTZ was a kind gift from Kobayashi Kako Co., Ltd. (Fuku) and was prepared at concentrations of 2.5, 5 and 10 mg/ml in physiological saline (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) after being dissolved in a small amount of 0.1 N HCl. Levallorphan tartrate (Lorphan injection) was obtained from Takeda Chemical Industries, Ltd. (Osaka, Japan) and was employed as the internal standard for detection by HPLC. [3H]Naloxone ([3H]NLX, specific activity 1961.0 GBq (53.0 Ci)/mmol) and unlabeled naloxone (NLX) were purchased from New England Nuclear Co. (Boston, MA, U.S.A.) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. All other chemicals and solvents were commercial products of analytical grade and were used without further purification.

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intraperitoneal anesthesia using urethane (ethyl carbamate, 1.3 g/kg, Sigma Chemical Co., St. Louis, MO, U.S.A.), polyethylene tubing (PE 50, i.d. 0.58 mm; o.d. 0.965 mm; Clay Adams, Parsippany, NJ, U.S.A.) was surgically implanted into the left femoral artery and the right femoral vein of the rats. After surgery, the animals were kept for at least 2 h before being used for the experiment, and body temperature was maintained at 35–37 °C using heat lamps during the experiment.

**Drug Administration and Sampling of Plasma** Three groups of rats (n = 3/group) were given PTZ i.v. at a dose of either 2.5, 5 or 10 mg/kg through the femoral cannula over a 10-s period. Frequent blood samples (200 μl) were taken through the arterial catheter at times of 2.5, 5, 10, 30, 60, 120, 180 and 300 min after the i.v. administration of PTZ. Plasma was separated from blood by centrifugation at 2000 × g for 5 min. Each plasma sample (100 μl) was stored at −20 °C until the next experiment.

**Analysis of PTZ in Plasma** The plasma concentration of PTZ was determined by a modification of the HPLC method as previously described. Briefly, 100 μl of a plasma sample was alkalinized by adding 20 μl of 1 M NaOH into micro-polypropylene test tubes, followed by the addition of 100 μl of the internal standard at concentrations of 0.1 μg/ml. The mixture was extracted with 600 μl of ethyl ether by mechanical shaking (Micro Tube Mixer MT-360, Tomy Seiko Co., Ltd., Tokyo, Japan) for 5 min. The tubes were centrifuged at 1500 × g for 10 min at 4 °C. The mixture was extracted again with 600 μl of ethyl ether. The organic phase was evaporated to dryness at 30 °C under a nitrogen stream. The dry residue was dissolved in 60 μl of the HPLC mobile phase and an aliquot of 20 μl was analyzed as the HPLC sample. For the calculation of unknown plasma samples, peak-area ratios for PTZ relative to the internal standard were used.

The HPLC system was constructed with a Model PU-980 intelligent HPLC pump (Jasco Co., Tokyo, Japan) and a Model FP-920 intelligent fluorescence detector (Jasco Co., Tokyo, Japan). A microprocessor (Model C-R3A Chromatopac, Shimadzu Co., Kyoto, Japan) was used for peak-area integration. The analytical column, Cosmosil Packed Column SC18-MS (150 mm × 4.6 mm i.d., particle size 5 μm, Nacalai Tesque Inc., Kyoto, Japan) was used at 25 °C. The mobile phase for assay consisted of acetonitrile and 0.05 M H₃PO₄ (20:80, v/v) at a flow-rate of 1.0 ml/min. The column elute was monitored fluorometrically at excitation and emission wavelengths of 278 and 324 nm, respectively. The retention times were 5.0 min for the internal standard and 7.4 min for PTZ. The time of detection was approximately 5 ng/ml of PTZ in plasma. The peak area ratios for PTZ relative to the internal standard were linearly related (r = 0.9995) to the amount of PTZ added to blank rat plasma in the range of 50 to 2000 ng/ml, and the mean analytical recovery of PTZ was 100.7 ± 6.7% in that range.

**Pharmacokinetic Analysis** The plasma concentration of PTZ was plotted against time after the i.v. administration of the drug. The time profiles of the disappearance of the 2.5, 5 and 10 mg/kg PTZ doses from the plasma were kinetically analyzed, based on the two-compartment open model using the nonlinear least-squares method (MULTI). The plasma concentration data were fitted into a two-exponential equation as follows:

\[ C_p = A e^{-\alpha t} + B e^{-\beta t} \]  

(1)

where \( C_p \) is the drug concentration in plasma at time \( t \), and \( A \) and \( B \) are the ordinate axis intercepts, and \( \alpha \) and \( \beta \) are the corresponding first-order disposition rate constants. The area under the plasma concentration-time curve (AUC), the total body clearance (CLtot), the steady state volume of distribution (\( V_{ss} \)) and the elimination half-life (\( t_{1/2} \)) of the β phase were calculated with the following relationships, independently:

\[ \text{AUC} = A/\alpha + B/\beta \]  

(2)

\[ \text{CL}_\text{tot} = D/(A/\alpha + B/\beta) \]  

(3)

\[ V_{ss} = A\beta^2 + B\alpha^2/(A\beta + B\alpha)^2 \]  

(4)

\[ t_{1/2} = 0.693/\beta \]  

(5)

where \( D \) is the i.v. dose administered.

**Determination of Receptor Occupation** The determination of receptor occupancy (RO) was assessed by use of the other group of rats and was performed as follows: basically according to the method described previously. Under light ether anesthesia, rats were cannulated with polyethylene tubing (PE 50) into the right femoral vein for drug injection. The cannula was routed subcutaneously in the dorsal and externalized on the dorsal side of the neck. The animals were left for at least 2 h after surgery to recover from anesthesia and were allowed to move freely before the injection. Rats were administered PTZ i.v. at a dose of either 2.5 or 10 mg/kg in the manner described above. Control animals were treated with vehicle solution (1 ml/kg). At each time point (2.5, 5, 10, 30, 60 and 120 min) after i.v. administration of the drug (\( n = 5 \)/group/time point) or vehicle (\( n = 5 \)/group/time point), rats were sacrificed by decapitation. Trunk blood was obtained to measure the plasma concentration of PTZ. Whole brain was removed, and the whole brain minus the cerebellum, brain stem and olfactory bulbs was used for the subsequent in vitro determination of the apparent number of drug-occupied receptors. In the present paper, we report the brain thus prepared simply as brain for convenience, although some regions described above are missing. The brain tissue, which weighed 1.2—1.3 g, was homogenized gently for 10 s in a Polytron homogenizer in 20 volumes of ice-cold 0.32 M sucrose and centrifuged at 1000 × g for 10 min at 4 °C. Pellets were discarded and supernatants were recentrifuged at 30000 × g for 20 min at 4 °C to produce a crude P2-synaptosomal fraction. The P2-synaptosomal fraction was resuspended in 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.7 at 25 °C; designated as standard buffer). Following incubation for 30 min at 0 °C, 0.4 ml of the P2-fraction suspension, 0.1 ml of [3H]NMDA (925 kBq/ml; 19 nM), 0.1 ml of unlabeled NLX (8 μM) or 0.1 ml of the standard buffer and 0.4 ml of the standard buffer with a final protein concentration of approximately 1 mg protein/ml were added to cold micro-test tubes. The protein concentration was determined according to the method of Lowry et al. with bovine serum albumin (BSA, Fraction V, Sigma Chemical Co., St. Louis, MO, U.S.A.) as a standard. Incubation for 60 min at 25 °C was terminated by filtration.
under low vacuum through Whatman GF/C glass fiber filters which were preliminarily moistened with ice-cold 50 mm Tris–HCl buffer (pH 7.7 at 25°C) containing 0.1% (w/v) BSA and 0.01% (w/v) Triton X-100.25) The filters were then washed three times with an additional 2.5 ml of ice-cold standard buffer and placed in scintillation vials. A counting solution (0.1 g of 1,4-bis-[2-(5-phenylloxazolyl)]benzene (POPOP), 4.0 g of 2,5-diphenyloxazole (PPO) and 500 ml of Triton X-100/1 of toluene25) in a volume of 10 ml was added, and the radioactivity on the filters was determined in a liquid scintillation counter (TRI-CARB 2000CA, Packard Instruments Co., Downers Grove, IL, U.S.A.). The subsequent procedure was the same as the sample assays described above, except that the filtration was performed by 0.4 ml aliquots in duplicate.

**Calculations** All binding was expressed as the specific binding calculated as the total binding minus the binding in the presence of unlabeled NLX (8 μM). The RO was calculated by the following equation according to the method described previously:23,24)

\[ RO(\%) = \left( \frac{R_S - R_A}{B_{max}} \right) \times 100 \]  

where \( R_S \) (fmol/mg protein) is the specific binding of \([H]^{3}NLX\) in the vehicle-treated rats, \( R_A \) (fmol/mg protein) is the specific binding of \([H]^{3}NLX\) in the PTZ-treated rats, and \( B_{max} \) (fmol/mg protein) is the maximum number of binding sites determined in vitro using separately prepared P2-fraction.

**[H]NLX Binding In Vitro** Intact rats were sacrificed by decapitation and a crude P2-synaptosomal fraction was prepared as described above. Then, 0.4 ml of the resuspended P2-fraction suspensions (1 mg protein/ml) containing various amounts of \([H]^{3}NLX\) (0.25—4 nm) in the presence and absence of unlabeled NLX (8 μM) were incubated for 60 min at 25°C, followed by filtration through Whatman GF/C glass fiber filters. The filters were washed three times with 2.5 ml of ice-cold standard buffer and the specific binding was determined as described above. The apparent dissociation constant \( (K_d) \) and \( B_{max} \) values for \([H]^{3}NLX\) binding were estimated by Scatchard analysis of the saturation data.

**RESULTS**

**Time Course of Plasma Concentration of PTZ** Figure 1 shows the plasma concentration-time profiles of PTZ after i.v. administration of three different doses (2.5, 5 and 10 mg/kg) of PTZ to rats. The plasma levels of PTZ declined two-exponentially with time, indicating that the two-compartment open model could be applied, as previously suggested for PTZ.2,3) The values of estimated pharmacokinetic parameters derived from the nonlinear least-squares regression program24) are listed in Table 1, showing a non-linear dependence of \( t_{1/2} \), \( V_{ss} \) and \( CL_{tot} \) with respect to dose.

**In Vitro [H]NLX Binding to the Brain P2-Fraction** As shown in Fig. 2(A), the specific binding of \([H]^{3}NLX\) (0.25—4 nm) to the P2-fraction, which was prepared by the same procedure as was employed in the study of receptor occupation, was saturable. Scatchard analysis of the data showed a linear plot with an apparent \( K_d \) value of 1.20 ± 0.04 nM and a \( B_{max} \) value of 206 ± 5.9 fmol/mg protein (Fig. 2(B)). The apparent \( K_d \) (1.2 nm) of \([H]^{3}NLX\) binding for the rat brain membranes prepared in the present study was in fairly good agreement with the previous value for that of 1.1 nm reported by Sato et al.25) The \( B_{max} \) value of the membranes, determined by \([H]^{3}NLX\) binding, was used in Eq. 6 to assess the RO in the in vitro experiment.

**Time Course of Receptor Occupation by PTZ** As shown in Fig. 3, time courses of the brain receptor occupation after the i.v. administration of two different doses (2.5 and 10 mg/kg) of PTZ was also assessed by use of the other group of rats. Depending on the pharmacological i.v. dose of PTZ, the receptor occupation decreased rapidly until 10 min, as the mean values, to 5% at the low dose (2.5 mg/kg) and to 14% at the high dose (10 mg/kg). The value of RO at the 10 mg/kg dose in the early time periods (2.5—10 min) was about 3 times higher than that of the 2.5 mg/kg dose. At each time point (2.5—120 min) in the RO experiment, the trunk blood was obtained to confirm the plasma concentration of PTZ. The plasma concentrations at each time point measured were consistent with the plasma concentrations at that time after the i.v. administration of PTZ (2.5 and 10 mg/kg), as indicated in Fig. 1.

**DISCUSSION** In order to trace the time course of drug action (or pharmacological effect), it is important to evaluate phar-
Fig. 2. Specific $[^{3}H]$NLX Binding to Synaptosomal Fraction of Rat Brain as a Function of Increasing Concentrations (0.25—4 nM) of Ligand (Panel A) and Its Scatchard Plots (Panel B).

Each point with a vertical bar and/or horizontal bar represents the mean ± S.E.M. of groups of three animals. In some cases, the S.E.M. values were smaller than the size of the symbols. Nonspecific $[^{3}H]$NLX binding was determined from the binding experiment in the presence of an excess amount of unlabeled NLX (8 μM) and was subtracted from the total binding. (B): $r = 0.9950$, $K_d = 1.29 ± 0.04$ nM, $B_{max} = 206 ± 5.9$ fmol/mg protein.

Fig. 3. Receptor Occupancy-Time Profiles by PTZ after i.v. Administration of PTZ to Rats.

Each point with a vertical bar represents the mean ± S.E.M. of groups of five animals at each time. Symbols: dose administered: ▲, 2.5 mg/kg; ○, 10 mg/kg. The RO was calculated by Eq. 6 from $B_{max}$ given in Fig. 2(B).

Pharmacokinetic processes such as metabolism, excretion, and distribution to major tissues which govern drug distribution in the body. Perhaps most important is the distribution process to the target organs where the pharmacological receptors exist. The subsequent pharmacodynamic process, which includes the interaction between drug and pharmacological receptors, reflects various pharmacokinetic and pharmacodynamic factors in vivo.

Receptor Occupation by PTZ. In the present study, we investigated the time profile of the receptor occupation by PTZ in the rat brain. To assess the in vivo opioid receptor occupation, we used an ex vivo method in which the measurement of specific $[^{3}H]$NLX binding in vitro after in vivo treatment with a pharmacological dose (2.5 or 10 mg/kg, i.v.) of PTZ was performed. PTZ is a mixed agonist-antagonist which acts as the agonist at the α opioid receptor and/or the antagonist at the μ opioid receptor.27

On the other hand, NLX is a typical opioid antagonist that is virtually devoid of agonistic activity and appears to be a competitive antagonist at several opioid receptors, including the μ and κ receptors.28 Although the ability of NLX to displace or prevent the binding of PTZ and to inhibit the analgesic activity of PTZ in the rat has been well documented,27,29 the $[^{3}H]$NLX concentration (19 nM) used in the in vitro binding assay caused hardly any dissociation of PTZ from the binding of PTZ to the specific opioid receptor sites by the in vivo treatment. Therefore, based on the ex vivo binding assay using $[^{3}H]$NLX, the brain RO by PTZ was measured accurately, as is clearly shown in Fig. 3. The time course of the receptor occupation, depending on the two pharmacological doses (2.5 and 10 mg/kg) of PTZ, decreased rapidly until 10 min. The results strongly suggest its fast binding kinetics: the association with and the dissociation from the specific opioid receptor sites in the brain, in addition to its fast rate of disappearance from the brain compartment. In our previous report, PTZ (2.5—10 mg/kg, i.v.) induced an increase in cerebral glucose utilization (cerebral neuronal
activity) within 10 min in the rat.\(^{13}\) This increase, which must have been due to the initial and direct interaction with specific opioid receptor sites in the central nervous system, thus, was supported by the RO in the early time periods after the i.v. administration of PTZ. It can easily be deduced that the RO measured in our ex vivo method includes the \(\kappa\) receptor acting as an agonist, which can participate in the analgesic effect, because we have already observed a sufficient emergence of analgesic effect after similar administration of PTZ (2.5—10 mg/kg, i.v.) to rats.\(^{13}\) The disappearance curve of receptor occupation by PTZ after 10 min was comparable with the decay of the analgesic effect of this drug in the mouse,\(^{41}\) rat\(^{13,14}\) and human,\(^{15}\) as reported previously. On the other hand, the lack of direct correlation of the time profile between the gradual increase of the analgesic effect\(^{13}\) and the rapid decrease of the receptor occupation was found at the early phase until 10 min after the i.v. administration of PTZ. To follow up on the delayed onset of the analgesic effect, the kinetic process of \(\kappa\) receptor occupation, excluding \(\mu\), may be important in estimating the agonistic action of PTZ at the \(\kappa\) opioid receptor.

The ex vivo method requires a receptor-containing preparation after the drug treatment in vivo; therefore, as suggested by Sawada,\(^{30}\) it is probable that during tissue homogenization and centrifugation, the drug which was bound to a receptor in vivo may partly dissociate from its binding sites, causing underestimation of the real in vivo occupation. Recently, Kotaki et al.\(^{31}\) have classified opioid agonists and antagonists based on the receptor occupancy in the brain calculated from a number of reports. They analyzed retrospective information on the mean plasma concentration and unbound fraction in humans after therapeutic dosing, as well as the binding affinity to \(\mu\) opioid receptors in the rat brain with the addition of sodium ions and without them, on eight opioid agonists (morphine, sufentanil, fentanyl, methadone, pethidine, levorphanol, buprenorphine and pentazocine) and two antagonists (naloxone and naloxone). The mean binding occupancy of PTZ (100 mg, p.o.) to the \(\mu\) opioid receptor has been estimated as 16.9\% with the addition of sodium ions (under a suitable in vivo condition) or 28.9\% without them. Judging from the fact that PTZ can also occupy the \(\kappa\) opioid receptor,\(^{27}\) the specific receptor occupation by PTZ (10 mg/kg, i.v.) in our results may be considered to represent the real in vivo occupation of the brain opioid receptors.

**Pharmacokinetics of PTZ.** The pharmacokinetic parameters of PTZ such as the \(t_{1/2}, V_{ds}\) and \(CL_{rat}\) were non-linearly dependent related to the three doses (Table 1). In accordance with our results, a non-linear dependence between dose and \(CL_{rat}\) was previously confirmed in rats.\(^{5}\) Ichimura et al. recently described the time course of blood and tissue concentrations of PTZ (0.5—10 mg/kg, i.v.) in the urethane-anesthetized rat according to a physiological pharmacokinetic model.\(^{5}\) Although the time course of the PTZ concentration in the blood obeyed a non-linear dependence on dose, the model successfully predicted the time course of the blood concentration and showed that the \(CL_{rat}\) was limited by the hepatic blood flow rate. In the present study, therefore, it is suggested that the change in \(CL_{rat}\) may also be due to a variation in the hepatic blood flow.\(^{32,34}\)

**Relationship between Receptor Occupation and Plasma Concentration of PTZ.** As shown in Fig. 1, the early time profile of plasma concentration at a distribution phase (x-phase) after i.v. administration of PTZ indicated 0.04 min\(^{-1}\) of the average rate constant at the x-phase, which was almost the same among the three dose levels. Following the i.v. administration of PTZ (10 mg/kg), a similar time course for disappearance has been reported in rat\(^{5,7}\) and mouse.\(^{4}\) It has already been reported that PTZ rapidly reaches the brain after parenteral administration\(^{4,9,35,36}\) or that the equilibrium in the rat is reached within 1 min between plasma and brain after i.v. administration\(^{5,7}\) due to PTZ’s high lipophilicity.\(^{9,37,38}\) No metabolites have been found in the brain after the parenteral administration of tritiated PTZ\(^{9,12}\) or of non-radioactive PTZ.\(^{4,35}\) In addition, the most direct evidence for the analgesia of PTZ has been considered to reside in the parent compound and not in its metabolites.\(^{11,36,39}\) Furthermore, in the rat, no significant change in plasma protein binding (46\%) or the blood-to-plasma concentration ratio (1.55) of PTZ with increasing blood levels was reported.\(^{30}\) In human, the ratio (0.2—0.6) of PTZ concentration in the cerebrospinal fluid-to-plasma reported by Agurell et al.\(^{3}\) agrees practically with the ratio (0.3—0.4) of that in unbound-to-total in plasma reported by Ehrenbo et al.\(^{40}\) We assumed in the rat that the unbound concentration of PTZ is the same between plasma and brain as in human, in which it can bind to the specific opioid receptors in the brain. The concentration of unbound drug in plasma (\(C_{p,f}\)) was calculated as follows:

\[
C_{p,f} = C_p \times f_{pu}
\]

(7)

where \(f_{pu}\) is the unbound fraction in plasma (0.54).\(^{5}\) Accordingly, the time course of \(C_{p,f}\) of PTZ predicted would be expected to correlate with that of the receptor occupation by PTZ until 120 min after the i.v. administration of PTZ to rats.

Under this assumption, the RO by PTZ was plotted against its \(C_{p,f}\) (ng/ml) according to Hill’s following equation:

\[
\log[B(B_{max} - B)] = n_H \log(C_{p,f}) - \log(K')
\]

(8)

where \(B\) (mol/mg protein) stands for the specific binding of PTZ calculated by \(R_{B} = R_{a}\) in Eq. 6 at the \(C_{p,f}\), \(n_H\) is the apparent Hill coefficient, and the \(K'\) is constant \((-K'[a^b + b^c + c^d + \cdots + z^z])\) composed of the interaction factors \((a, b, c, \cdots, z)\) for more than one drug molecule for several binding steps and the intrinsic association constant \((K')\). As shown in Fig. 4, the time-dependent change in the receptor binding of PTZ in relation to the \(C_{p,f}\) at various times (2.5—120 min) after the i.v. administration of two different doses (2.5 and 10 mg/kg) was analyzed by least square analysis. A good correlation \((r = 0.8650)\) was observed between the receptor occupation and \(C_{p,f}\) of PTZ. The apparent Hill coefficient \((n_H)\) of this drug was 1.34. In this analysis, it was assumed that the \(C_{p,f}\) of PTZ is the same between plasma and brain. However, in Eq. 8, even if the concentration of unbound drug in brain \((C_{br,f})\) calculated from the unbound fraction in the brain
(f_{br,u}) and the C_{p,f} is substituted for the C_{p,t}, the n_{H} still does not change except for the K'. Although the basic form of interaction with n_{H}=1 (non-cooperativity) is a one-to-one reaction between a drug molecule and a receptor to form a drug-receptor complex, secondary responses with the same drugs yield n_{H}≠1 (cooperativity), usually with non-integral values. Besides, this n_{H} value (1.34) is often confused with the number of ligand-binding sites, but the two quantities in Eq. 8 are related only by the restriction that the Hill coefficient (n) cannot exceed the real number of sites. Consequently, the n in this analysis is virtually 2, which suggests that the specific PTZ binding in the present study did not represent binding to a single population among the opioid receptors but was the binding between at least to two different populations, the µ opioid receptor and the κ opioid receptor, among them. Moreover, the analysis demonstrated that the C_{p,t} of PTZ was 2.3 μg/ml (8.1 μM), which is necessary to occupy 50% of the total number of opioid receptors in the brain (E_{C_{50}}) when [B/(B_{max}−B)]=1. This must have occurred within 2.5 min in Fig. 3. At the present time, it is unclear to us how the E_{C_{50}} analyzed affects subsequent pharmacodynamic responses, including the increase in cerebral glucose utilization and the delayed onset of the analgiesic effect in the early time periods after the i.v. administration of PTZ.

In conclusion, we characterized the time profile of receptor occupancy by PTZ in the rat brain using the ex vivo method. After i.v. administration of PTZ, the time course of the brain RO decreased rapidly until 10 min in a dose-dependent manner. The results strongly suggest rapid binding kinetics of PTZ’s association with and dissociation from the specific opioid receptor sites in the brain in addition to its fast rate of disappearance from the brain compartment. Furthermore, we were able to evaluate the relation among the time course of unbound concentration in plasma and that of the specific occupation in the brain opioid receptor until 120 min after the i.v. administration of PTZ to rats.

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