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BRAIN REGIONAL PHARMACOKINETICS OF BIPERIDEN IN RATS

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ABSTRACT

The pharmacokinetic profiles of biperiden (BP) in blood and in specific brain regions were investigated in rats after acute i.v. administration. The regional brain-to-blood unbound concentration ratios (K_{pf}) were also determined after 16 h intravenous infusion of BP. The K_{pf} values ranged from 30 to 75 in the different brain regions and showed decreasing concentrations in the following order: pons + medulla oblongata, basal ganglia, amygdala, hypothalamus, thalamus, mesencephalon, bulbus olfactorius + septum, hippocampus, frontal cortex, occipital cortex, cerebellum. The relationship between BP and acetylcholine (ACh) concentrations in the brain regions was examined. ACh levels in the various brain regions ranged from 8 to 44 ng g^{-1} tissue. There was a significant correlation between the K_{pf} values of BP and the levels of ACh in the brain regions except for the pons + oblongata. BP concentrations in the brain regions after BP administration were predicted based on the physiological pharmacokinetics. There was reasonable agreement between the model predictions and the observed data.

KEY WORDS Biperiden Regional brain distribution Acetylcholine Choline Rats

INTRODUCTION

Biperiden (BP) is widely used as an anticholinergic drug to treat parkinsonian syndrome and neuroleptic-induced akathisia.[1] Although there have been several reports on the pharmacokinetics of BP in healthy human volunteers,[2,3] and patients,[4] relatively little information regarding the relationship between the pharmacological response and plasma concentration is available.

In order to predict the pharmacological efficacy from the plasma concentration, it is preferable to know the drug concentration at the effector site rather than in whole tissue. The brain is composed of various tissues, to which some drugs have been reported to be unevenly distributed.[5,6] BP is a potent anticholinergic drug, which is thought to exert its central effect by blocking brain muscarinic cholinoceptors which are located mainly in the medulla oblongata.[7] Acetylcholine (ACh) in the brain is also unevenly distributed.[8] To evaluate the pharmacological response *in vivo*, it is important to know the regional

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brain distribution of BP in relation to the levels of ACh and choline (Ch). However, the regional brain distribution of BP is still unknown.

The purpose of this paper is to investigate the regional brain distribution and pharmacokinetics of BP in rats. This paper also reports the relationships between the levels of ACh, Ch, and BP in the various regions of the brain.

MATERIALS AND METHODS

Materials

BP (Dainippon, Osaka, Japan) was used as supplied. Monoethylhomocholine was kindly supplied by Eikom Co. (Kyoto, Japan). All other chemicals were of reagent grade and were used without purification.

Determination of serum protein binding

The extent of drug binding to rat serum protein was measured by the equilibrium dialysis technique with a sample volume of 0·8 ml as described previously.[4]

Animal experiments

Male Wistar rats (Sankyo Laboratory Animal Co., Toyama, Japan) were used randomly in this study. Animal experiments were carried out in essentially the same way as described previously.[9] Briefly, under light anesthesia the femoral artery and vein were cannulated with polyethylene tubing. Surgery was completed within 30 min. The cannulated rats were kept on restraining plates. No anesthesia was used in the subsequent stages. To determine the tissue-to-plasma partition coefficient in the steady-state, infusion studies were performed to obtain plasma concentrations ranging from 100 to 200 ng ml^{-1}. The priming and maintenance doses were calculated based on preliminary determination of the values of the distribution volume and clearance of the drug after i.v. bolus administration. BP was infused at a rate of 10 $\mu g\ min^{-1}\ kg^{-1}$ after intravenous bolus injection of the priming dose of 3·2 mg kg^{-1}, since the volumes of distribution and clearance in rats were 14·0 l kg^{-1} and 67·7 ml $min^{-1}\ kg^{-1}$, respectively.[10] At 16 h after the infusion studies began, the rats were sacrificed for tissue sampling, and the brains were dissected according to Gispen *et al.*[11] into the following regions as soon as possible: bulbus olfactorius + septum (BO.S), basal ganglia (BG), frontal cortex (FC), hypothalamus (HT), thalamus (TL), cerebellum (CE), pons + medulla oblongata (P.MO), mesencephalon (MS), hippocampus (HC), amygdala (AM), and occipital cortex (OC). The procedure used for preparing tissue homogenate was essentially the same as that described previously.[12] The plasma was separated by centrifugation and stored at −30°C until assayed.

For a study of Ch and ACh distributions in the brain, two experimental groups of rats, one administered and one not administered BP, were used.

The former group of rats received an intramuscular injection of BP (3·2 mg kg^{-1}) 2 h prior to dissection. Both groups of rats were killed by focusing a beam of microwave radiation (3·0 kW at 2·45 GHz, Gerling-Moore Metabostat System; Gerling-Moore, Santa Clara, CA, USA) on the head for 2·5 s. Then the brains were dissected as described above.

Assay for BP

Drug concentrations in plasma and various brain homogenates were determined by gas liquid chromatography (GLC) as described previously.[12] Briefly, homogenized tissues and/or plasma were mixed with an equal volume of 1 M Na_2CO_3 buffer (pH 10·0), an internal standard was added, and samples were extracted with diethyl ether. The samples were centrifuged for 5 min at 2000 rev min^{-1}, and then the organic phase was transferred into another series of test tubes, each containing 2 ml of 1 N HCl. The drug was re-extracted into the HCl, and the resulting ether phase was discarded. Then 1 ml of 3 N NaOH was added to the aqueous phase, together with 5 ml of diethyl ether. The final ether phase was transferred to a glass centrifuge tube, and evaporated. The dried residue was dissolved in 20 μl of diethyl ether, and most of the solution was applied to the chromatograph.

Assay for Ch and ACh

After the rats were killed by focused microwave radiation, the brains were dissected according to Gispen *et al.*[10] as soon as possible. ACh and Ch concentrations in various brain homogenates were determined by the method of Barnes *et al.*[13] Briefly, the dissected brains were immediately homogenized in 500 μl per 100 mg tissue of 0·2 M perchloric acid containing 10 nmol monoethylhomocholine with an ultrasonic homogenizer for 30 s. The homogenates were stored in an ice-cold bath for 30 min to remove the protein completely, and then centrifuged for 15 min at 2000 g and 4°C. Then 200 μl of the supernatant was transferred into another series of test tubes, each containing 200 μl of 0·2 M $KHCO_3$. After the tubes were stored for 30 min in an ice-cold bath, the aqueous phase was filtered with a 0·45 μm filter and 20 μl of the solution was injected into the HPLC. Calibration curves were obtained by the same method for each biological sample. The detection of limits of the method for ACh and Ch for the homogenized samples were both 0·625 nmol, and the coefficient of variation was less than 10 per cent.

HPLC system

For the determinations of ACh and Ch, a HPLC (Shimadzu LC-6A) was equipped with an electrochemical detector (Eikom, ECD-100). An analytical column (Eikom, MA-ODS, 50 ×4·6 mm i.d.) was used for the separation of

Ch, monoethylhomocholine, and ACh. The enzyme column (40 × 4·6 mm i.d.) containing covalently bound choline oxidase and acetylcholinesterase was inserted between the analytical column and the detector. The voltage applied to the platinum electrode was 250 mV (versus the solid state reference electrode incorporated into the analytical cell) and the detector signal was monitored using an integrator (Shimadzu Chromatopac C-R3A).

Data analysis

Area under the plasma concentration versus time curve (AUC) was estimated using the trapezoidal rule. AUC was extrapolated to infinite time from the last determined plasma concentration by using the terminal slope of the log plasma concentration–time curve. Initial uptake clearance of BP into the brain was determined as the ratio of BP amount in the regional brain at 2 min to area under the plasma concentration versus time curve from 0 to 2 min (AUC_{0-2}).

The model prediction using differential equations was performed using a perviously described program.[4,9] A Facom M360AP digital computer at the Data Processing Center, Kanazawa University was used.

The measure of the fit between the observed (C_{obs}) and the predicted (C_{pred}) concentrations of BP was based on the coefficient of determination, r^2, calculated from the equation: $r^2 = 1 - \Sigma dev^2/Sy^2$, where $Sy^2 = \Sigma obs^2 - (\Sigma Y_{obs})^2/n$, $dev^2 = (Y_{obs}-Y_{pred})^2$, and n represents the number of determinations. In this calculation, the logarithmic values of C_{obs} and C_{pred} were employed as Y_{obs} and Y_{pred}.[14]

RESULTS

The regional brain-to-plasma unbound concentration ratios (K_{pf}) at steady-state

After the drug was infused intravenously for 16 h at a constant rate, the K_{pf} values for various brain regions were calculated from the steady-state concentrations in the tissue and plasma. K_{pf} is defined as the ratio of the concentration in the tissue (ng g^{-1} tissue) to the concentration in the plasma (ng ml^{-1}). As illustrated in Figure 1, the K_{pf} values differed among the brain regions ranging from 30 to 75. The highest level of BP was found in the P.MO, the lowest in the CE. The concentrations in the different regions decreased in the following order: P.MO, BG, AM, HT, TL, MS, BO.S, HC, FC, OC, CE. A significant difference was not observed in K_{pf} from BG to FC. However, there was a significant group difference in the 10 regional (except for P.MO) BP concentrations by ANOVA ($F = 5{\cdot}470$, $p < 0{\cdot}001$).

ACh and Ch levels in different areas of the brain

The relationships between the levels of ACh, Ch, and BP in the various regions of the brain were examined in the control and BP treated rats. Table

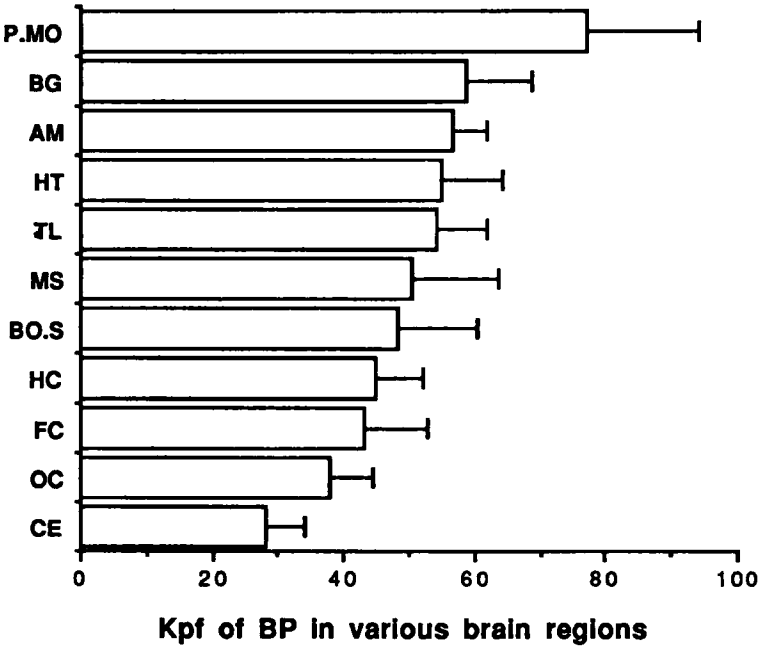

Figure 1. Regional distribution of BP in rat brain after continuous intravenous infusion for 16 h. BP was infused at a rate of 10 μg min^{-1}kg^{-1} after intravenous bolus injection of the priming dose of 3.2 mg kg^{-1}

Table 1. ACh and Ch concentrations in different regions of the rat brain with or without BP treatment. A group of rats was treated with an intramuscular injection of BP (3.2 mg kg^{-1}) 2 h prior to dissection

	Ch (nmoles 100 mg^{-1} protein)		ACh (nmoles 100 mg^{-1} protein)	
	Control ($n = 11$)	+ BP ($n = 6$)	Control ($n = 11$)	+ BP ($n = 6$)
AM	42·2 ± 25·9	36·0 ± 15·3	38·3 ± 8·9	29·1 ± 5·3
BG	23·3 ± 5·8	27·2 ± 9·2	43·9 ± 9·3	38·8 ± 7·2
BO.S	41·6 ± 26·8	43·7 ± 20·3	23·3 ± 8·4	26·3 ± 17·0
CE	30·4 ± 8·4	37·2 ± 10·5	7·7 ± 3·3	7·7 ± 1·5
FC	28·4 ± 6·7	30·6 ± 7·5	26·8 ± 6·2	21·0 ± 4·1
HC	25·9 ± 6·4	29·5 ± 11·9	24·7 ± 5·7	22·5 ± 3·4
HT	22·5 ± 9·3	26·9 ± 11·3	26·7 ± 5·8	28·9 ± 8·2
MS	24·2 ± 7·7	23·5 ± 7·8	33·2 ± 9·4	32·7 ± 4·2
OC	27·9 ± 6·1	31·1 ± 10·5	15·6 ± 3·4	12·5 ± 1·9
P.MO	32·3 ± 8·0	29·0 ± 9·6	26·7 ± 7·9	22·4 ± 5·6
TL	18·4 ± 9·1	22·9 ± 11·9	31·4 ± 9·1	35·4 ± 14·1

Mean ± SD.

1 shows the levels of ACh and Ch in 11 areas of the brain. No significant differences in the Ch levels were observed among these regions. The levels of ACh ranged from 7·7 ± 3·3 to 43·9 ± 9·3 nmol g^{-1} tissue as listed in Table 1. BP showed no effect on the levels of Ch in the various regions of the brain. BP also showed no effect on the levels of ACh in 10 of the regional brain areas. However, in the AM (Table 1), there was a significant effect of BP administration on the ACh level by ANOVA (F= 5·14, $p<0·039$). The level of ACh decreased significantly with BP administration ($p<0·05$ by t-test).

Relationships between ACh and BP levels in different areas of the brain

As shown in Figure 1, the values of K_{pf} differed in various regions of the brain. In order to evaluate the pharmacological response *in vivo*, we examined the relationship between the levels of BP and ACh, which may compete for receptor binding. Figure 2 shows the correlation between the K_{pf} of BP and

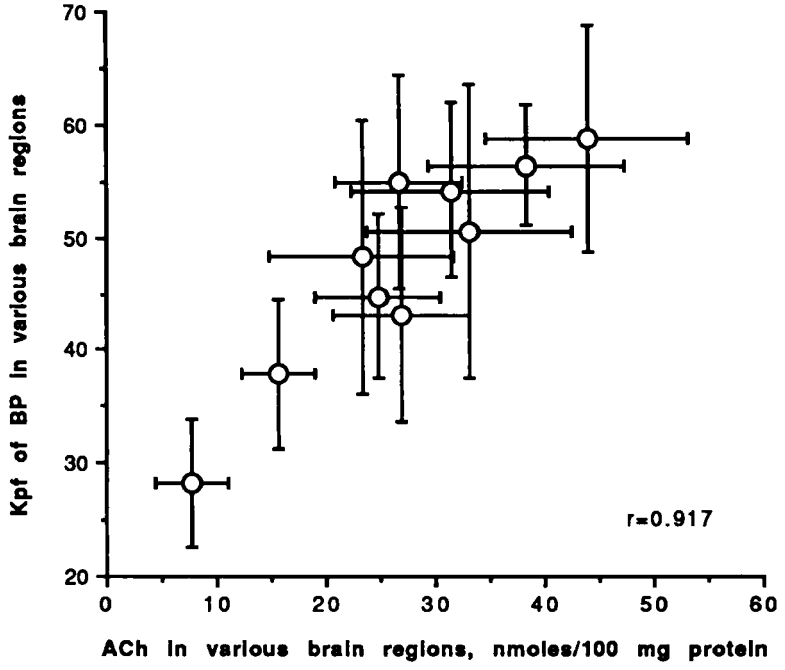

Figure 2. Relationship in various regions of the rat brain between tissue-to-plasma partition coefficients of the un-ionized form of the drug (K_{pf}) and the ACh concentrations. R represents the correlation coefficient except for pons + medulla oblongata

ACh levels in 10 portions of the brain except for P.MO. There was a linear relationship with a correlation coefficient of 0·918. In the case of P.MO, the K_{pf} was two-times higher than that of FC in which the ACh level was almost the same level.

Model development and predicted time course of BP concentrations in plasma and brain

As previously reported,[4,10,15,16] we established a mathematical model to extrapolate the tissue distribution kinetics of BP into anatomically distinct tissues. This physiological model for BP was applied to the present study incorporating three regional brain portions, such as P.MO, FC, and CE. The regional K_{pf} values determined in the above section (Figure 1) were used for the prediction together with the measured tissue volume in the present study and the previously reported blood flow rate.[17] The physiological parameters are listed in Table 2. The pharmacokinetic profiles for BP in blood and in specific brain

Table 2. Physiological and pharmacokinetic parameters of BP

Regions of brain	V_t* (ml)	Q_t† (ml $min^{-1}g^{-1}$)	K_{pf}*
P.MO	0·21	1·87	78·2
FC	0·30	1·39	44·1
CE	0·23	1·00	28·4

*Determined in the present study.
†See Sakurada *et al.*[17]

regions were examined in rats after acute i.v. administration. Figure 3 shows the calculated variations in BP concentrations in the plasma, P.MO, FC, and CE at a dose of 3·2 mg kg^{-1} following 2 min infusion. The predicted curves were in reasonable agreement with the mean values of the observed data, having coefficients of correlation in the range of 0·992 and 0·977.

DISCUSSION

The present study was designed to determine the changes in BP concentrations in various regions of the brain *in vivo* in relation to ACh levels in the corresponding regions. To predict the concentrations of BP after BP administration, we attempted to construct a physiologically based pharmacokinetic model in separated regions of the brain for P.MO, FC, and CE using each K_{pf}, Q_t, and V_t. As shown in Figure 3, there was reasonable agreement between the model predictions and the observed data.

After i.v. administration, BP crossed the blood-brain barrier rapidly with maximal brain concentrations reached 3–10 min after dosing. The cerebellar level of BP at 3 min after the dosing was 10 times higher than the plasma level. By considering the blood-to-plasma concentration ratio (=1·15)[16] and the brain regional blood flow rate,[17] the maximum uptake clearance limited by the blood flow rate was calculated to range from 1·15 to 2·15 ml min^{-1} g^{-1}. From the results in Figure 3, the uptake clearance (calculated as BP amount

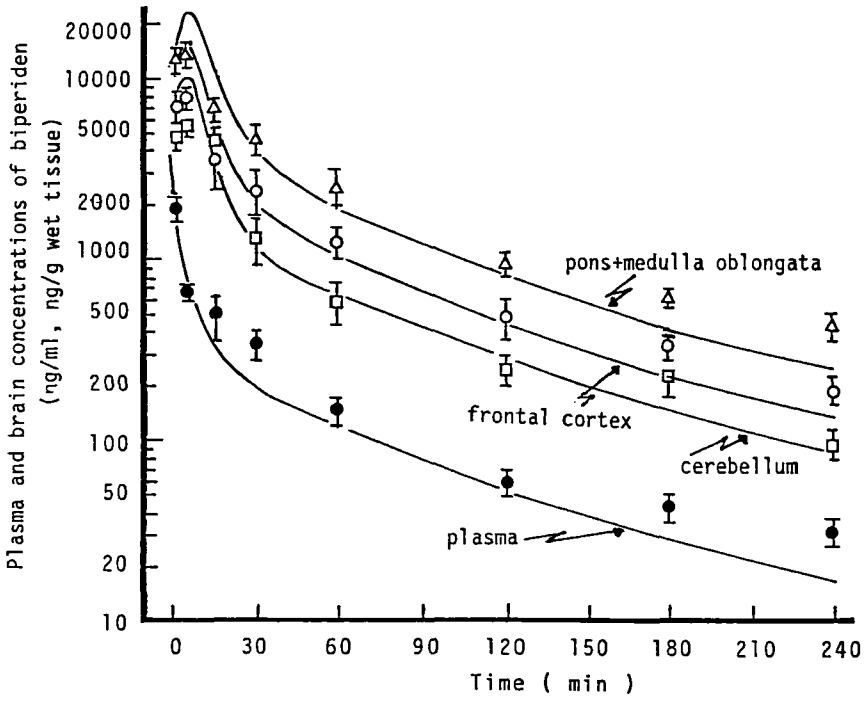

Figure 3. Model-predicted (lines) vs observed (points) concentrations of BP in plasma, pons + medulla oblongata, frontal cortex and cerebellum after a 3.2 mg kg^{-1} intravenous 2 min infusion into rats. Each point represents the mean of three rats, and bars represent the standard errors of the mean

in the regional brain at 2 min after the administration divided by $AUC_{0\text{-}2}$) ranged from 3 to 6 ml/min^{-1} g^{-1} in the FC, CE, and P.MO. These clearance values exceeded the maximum uptake clearance limited by the blood flow rate. Strictly speaking, these local blood flow rates were determined by iodo [^{14}C]antipyrine.[17] The tissue concentrations were measured by a quantitative autoradiographic technique which allowed precise localization in the various structural components of the brain.[17] In this study, we determined brain BP concentrations by the tissue sampling method. The whole brain blood flow rate using the method of tissue sampling after the exhalation of ^{133}Xe were reported as 0·98 ml min^{-1} g^{-1}.[18] Although use of the latter blood flow rate may be more appropriate, no adequate explanation can be given of these high clearance values. Since the measurement of concentrations in the plasma and brain in the very early phase after the administration is fraught with error, these values should be considered only rough estimates. The uptake of BP into the brain is probably blood flow rate limited.

The ratio of AUCs of FC, CE, and P.MO to plasma concentrations of

BP have been estimated to be 6·6, 7·4 and 12·4, respectively, which are close to the values of the K_{pf} of BP determined by steady-state infusion studies. The order of maximal concentrations of BP in the various regions of the brain also closely corresponded to that of K_{pf}.

The usefulness of routine monitoring of antipsychotic drug plasma levels has not yet been established.[19] The interaction of BP with brain muscarinic cholinoceptors has been reported in *in vitro* receptor binding studies;[20] however, the interaction of BP with *in vivo* cholinoceptors and the neurotransmitter, ACh, has not yet been reported. In the present study, ACh levels in the various brain regions ranged from 7·7 to 43·9 ng g^{-1} tissue. It has been reported that ACh is distributed throughout the central nervous system, with high concentrations found in the cerebral cortex (corresponding to FC in the present study), thalamus (TL), and various nuclei in the basal forebrain (BG).[7] This distribution is essentially in agreement with the present results. As shown in Figure 1, the values of the K_{pf} of BP at steady-state differed among the various regions of the brain. The highest K_{pf} was obtained in P.MO. Since P.MO is a lipid-rich region,[21] BP may distribute according to the lipid content of the brain because of its high lipophilicity.[22] When the region of P.MO was excluded, there was a significant correlation between the K_{pf} values of BP and the levels of ACh in 10 regions. Whether the similar distribution of BP and ACh in the brain accounts for the favorable pharmacological response of BP remains to be clarified. However, several possibilities may explain the mechanism of similar distribution. It is known that the distribution of acetylcholine in the central nervous system parallels that of choline acetyl transferase and ACh.[7] Moreover, spheroid vesicles contain acetylcholine at the end of synaptic junctions. BP may bind to some of these coextensive with acetylcholine. Recent pharmacokinetic studies in our laboratory have demonstrated that the distribution volume of BP is greatly influenced by small changes in the volume of fat tissue.[4,10,15,16] The tissue-to-plasma unbound concentration ratios in different tissues ranged from 16 to 445,[4] with high ratios obtained in the lung and fat. The values of some viscera, such as brain, heart, kidney, and gut, were in the range of 50 to 100. Although the coincidence of each tissue-to-plasma concentration ratio in whole tissue in rats and rabbits was confirmed, the factors accounting for tissue high affinity remain to be clarified. In the present study, we found an uneven distribution of BP in the brain, although the factors responsible for this are not known. Further detailed experiments using brain subcellular fractions are now under way in our laboratory.

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