Characteristic Subcellular Distribution, in Brain, Heart and Lung, of Biperiden, Trihexyphenidyl, and (−)-Quinuclidinyl Benzylate in Rats

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The subcellular distribution of biperiden (BP), trihexyphenidyl (TP) and (−)-quinuclidinyl benzylate (QNB) in brain, heart and lung following high dose (3.2 mg/kg) i.v. administration was investigated in rats. The subcellular distribution of BP or TP used clinically conformed with that of QNB, a typical potent central muscarinic antagonist. The concentration–time courses of the brain subcellular fractions for these drugs were of two types which decreased slowly and in parallel to the plasma concentration. The subcellular distribution in the brain and heart was dependent on the protein amount of each fraction. The percent post-nuclear fraction (P2) of the total concentration in the lung was characteristically about 3–5 times larger than that in the heart. It was elucidated that the distribution in the lung differs from that in the brain and heart, with high affinity which is not dependent on the protein amount in the P2 fraction containing lysosomes. On the other hand, at a low dose (650 ng/kg) of 3H-QNB, each fraction as a percentage of the total concentration in the brain increased in synaptic membrane and synaptic vesicles and decreased in nuclei and cytosol as compared with the high dose. These results show that although the tissue concentration–time courses of anticholinergic drugs appear to decrease simply in parallel to plasma concentration, the subcellular distribution exhibits a variety of patterns among various tissues.

Key words anticholinergic drug; subcellular distribution; lysosome; biperiden; trihexyphenidyl; quinuclidinyl benzylate

In order to evaluate the effectiveness and side effects of therapeutic drugs, we have studied distribution kinetics and their variations. Biperiden (BP) and trihexyphenidyl (TP) have been widely used as anticholinergic drugs to treat Parkinsonian syndrome and neuroleptic-induced akathisia. The distribution kinetics of these drugs include a large distribution volume and a different tissue-to-plasma concentration ratio (Kd) among various tissues.1,2 It was elucidated that lysosomes have the highest affinity for basic drugs in the rat liver, and their contribution to subcellular distribution is dependent on the intralysosomal pH, which is also affected by these drugs.3

(−)-Quinuclidinyl benzylate (QNB) is known to be a typical potent central muscarinic antagonist which labels the cholinergic receptor in rat brain. We reported that there was a difference in the distribution kinetics after the i.v. administration of low dose (325 ng/kg) and high dose (3.2 mg/kg) QNB in rats.4 Namely, after administration of the high dose, the tissue concentration–time course decreased parallel to the plasma concentration, while after the low dose, QNB accumulated in the brain and heart.

On the other hand, to examine the appearance of drug effectivenes and side effects, it is essential to elucidate not only tissue distribution but also subcellular distribution. Although the subcellular distribution of basic drugs has been reported, more information is required. Moriyama et al. reported that brain synaptic vesicles accumulate neuron blockers, chlorpromazine and haloperidol, against concentration gradients of more than 100-fold in the presence of ATP.5 Pulmonary toxicity coincides with an intralysosomal accumulation of amidarone in the lung and may be causally related to the drug-induced intracellular storage of phospholipids.6,7 Friedman and Cooper reported that the rat brain subcellular distribution of clomipramine and its metabolite was similar regardless of administration terms.8 Therefore, since basic drugs are distributed widely over not only the target organ but also the total body, it is necessary to elucidate differences in their subcellular distribution among various tissues. Moreover, to evaluate the clinical pharmacological response, it is preferable to know the subcellular pharmacokinetics of anticholinergic drugs on the effector sites such as brain synaptic membrane and synaptic vesicles.

The purpose of this paper is to study the characteristic subcellular distribution of anticholinergic drugs in the brain, heart and lung of rat with a changing dose.

MATERIALS AND METHODS

Materials BP (Dainippon Pharmaceutical Co., Osaka, Japan) and TP (Nippon Lederle Co., Japan) were used as supplied. QNB was purchased from RBI Research Biochemicals, Inc.(MA). 3H-QNB (41.6 Ci/mmol) was purchased from Amersham International, Ltd. (Bucks, U.K.). All other chemicals were of reagent grade and were used without purification.

Animal Experiments Male Wistar rats (255±21 g; mean±S.D., Toyama, Japan) were used randomly in this study. Animal experiments were carried out essentially as described. Briefly, the dose used in this study, 3.2 mg/kg or 650 ng/kg, was chosen based on previous studies. A 0.24 ml portion of each drug solution was injected through the left carotid vein. Blood samples were withdrawn from the right carotid vein at designated time intervals, then collected in heparinized tubes. Plasma was separated by centrifugation and stored at −30°C. To determine the tissue subcellular fraction-to-plasma concentration ratio in the steady state (Ksub), the femoral vein was cannulated with polyethylene tubing under light anesthesia. The infusion studies were performed at a rate of 0.765 ml/h (5.32 mg of drug solution/ml)

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after i.v. bolus injection of a priming dose of 3.2 mg/kg. At 12 h after the infusion studies began, and the rats were sacrificed for tissue sampling.

Assay for BP, TP and QNB The drug concentrations of BP, TP and QNB in plasma and various subcellular fractions were determined using gas–liquid chromatography as described. The radioactivity of 2H-QNB in various subcellular fractions was determined in 10 ml of scintillation fluid with a liquid scintillation counter by a method described. Metabolites of 2H-QNB in tissues after the separation by HPLC were present only in negligible amounts.

Assay for Protein Protein concentration was determined using a commercial Kit (Protein Assay Kit, Bio-Rad Laboratories, Ltd., Osaka, Japan).

Subcellular Fractionation The brain, heart and lung of the rats were fractionated according to de Robertis et al. Briefly, the rats were sacrificed for tissue sampling, then the excised tissue was homogenized with ten volumes of ice-cold 0.32 M sucrose. All subsequent steps were performed at 4°C. To obtain the nuclei fraction (P1), the homogenate was centrifuged at 10000×g for 10 min. The resulting post nuclear supernatant was centrifuged again at 12300×g for 20 min to obtain the post-nuclear fraction (P2). Then, the supernatant was centrifuged at 100000×g for 60 min to obtain the microsome fraction (P3) and the supernatant (cytosol, S1).

The P2 of brain was resuspended by osmotic shock and centrifuged at 20000×g for 20 min to obtain a pellet (P3). Thereafter, the supernatant was centrifuged at 100000×g for 60 min to obtain the synaptic vesicles (P4) and the supernatant (S2). The P3 was resuspended and layered on a density of 0.32, 0.8, and 1.2 M sucrose, then centrifuged at 50000×g for 120 min. The gradients were separated into 3 fractions. The order of the light fraction is as follows: myelin (P31), synaptic membrane (P32), and mitochondria (P33).

RESULTS

The concentration–time courses of the plasma and brain subcellular fractions in rats 5, 20 and 120 min following 3.2 mg/kg i.v. administration of BP, TP and QNB are shown in Fig. 1. The concentration–time courses of the brain subcellular fractions for these drugs decreased roughly parallel to the plasma in P1, P2 and P3, but slowly in P33 and P32.

Figure 2 shows the relationship of the brain subcellular distribution between BP or TP and QNB at 20 min. These data were obtained from Fig. 1. There were two good correlations for BP and TP (r = 0.885 and 0.950, respectively). The concentrations of BP and TP in each fraction were about 4 times higher than that of QNB.

The concentrations of BP, TP and QNB in subcellular fractions of the lung and heart at 20 and 120 min after a 3.2 mg/kg i.v. administration in rats are shown in Fig. 3. The total concentrations of these drugs in the lung was about 10 times higher than those in the heart. The values for P2 as a percentage of the total concentration in the heart at 20 min for each drug were about 10% and did not vary until 120 min. On the other hand, those for P3 in the lung at 20 min
were about 35%, significantly higher than in heart. Moreover, with time, the values for $P_2$ in the lung at 120 min increased characteristically to about 55%.

Figure 4 shows the concentration of each brain subcellular fraction of $^3$H-QNB at 5 and 20 min and at 24 h following 650 ng/kg i.v. administration in rats. It was observed that the QNB concentrations of most fractions tended to increase gradually and then accumulated during the 24 h.

Figure 5 illustrates the relationship between each subcellular fraction as a percentage of the total concentration of QNB in the brain at 20 min after the i.v. administration of 3.2 mg/kg and at 24 h after the i.v. administration of 650 ng/kg. There was a marked difference in the brain subcellular distribution of QNB among these cases. $P_1$, $P_3$, $S_1$, and $S_2$ have a high affinity for QNB in the brain subcellular fraction at a high dose, whereas $P_{12}$, $P_3$, and $P_4$ have a high affinity at a low dose.

Figure 6 shows the concentrations of $^3$H-QNB in each subcellular fraction in the heart and lung at 5 and 20 min and at 2 h after 650 ng/kg i.v. administration. The total concentration of $^3$H-QNB in the heart decreased temporally at 20 min and then increased at 2 h, both significantly, as compared with the values at 5 min. On the other hand, the total concentration of $^3$H-QNB in the lung decreased gradually, reaching less than 10% at 2 h compared to the total concentration at 5 min. The values for $P_1$, $P_3$, $P_5$, and $S_1$ as a percentage of the total concentration of $^3$H-QNB in the heart at 5 min were 33, 31, 28 and 8%, respectively. Similarly, the percentages for fractions in the lung at 5 min were 1, 48, 10 and 41%, respectively. There was a characteristic difference in the pattern of the subcellular distribution of $^3$H-QNB between the heart and lung. In the heart, $^3$H-QNB concentrations in $S_1$ were about a quarter of those in other fractions, whereas in the lung, $^3$H-QNB was distributed in the $P_1$ fraction and in $S_1$, but was distributed very little in $P_1$.

Figure 7 illustrates the relationships between the protein contents and the values of $K_{eq}$ obtained from the BP infusion study. There were good correlations in the brain and heart ($r=0.916$ and 0.917, respectively). In the lung, there was no correlation between these fractions ($r=0.06$); however, with the exception of the $P_2$ fraction, a good correlation was obtained ($r=0.987$).

![Graph showing the relationship between protein content and $K_{eq}$ values in various subcellular fractions](image)

Fig. 2. The Relationship of the Brain Subcellular Concentration between BP or TP and QNB at 20 min after 3.2 mg/kg i.v. Administration in Rats

The dotted line represents the regression for BP and QNB ($\bullet$). The solid line represents the regression for TP and QNB ($\circ$). Each point represents the mean ± S.E.M. of three experiments.

![Bar charts showing concentrations of BP, QNB, and TP in heart and lung fractions](image)

Fig. 3. The Concentration of Each Subcellular Fraction in the Heart and Lung at 20 and 120 min after 3.2 mg/kg i.v. Administration of QNB, BP and TP in Rats

Each bar represents the mean ± S.E.M. of three experiments. ■, heart at 20 min; ◊, heart at 120 min; □, lung at 20 min; ◊, lung at 120 min.
DISCUSSION

In the present study, we determined that there are characteristic differences in the subcellular distribution of anticholinergic drugs among the brain, heart and lung in rats. Furthermore, we investigated the relationship between the subcellular distribution at the high dose used clinically and at the low dose used for receptor binding studies using QNB.

Fig. 4. The Concentration of \(^{3}H\)-QNB in the Brain Subcellular Fraction at 5 min, 20 min and 24 h after 650 ng/kg i.v. Administration for \(^{3}H\)-QNB in Rats
Each bar represents the mean±S.E.M. of three experiments. ■, 5 min; □, 20 min; □, 24 h.

Fig. 5. The Relationship between Each Subcellular Fraction as a Percentage of the Total Concentration of QNB in the Brain at 20 min after i.v. Administration of 3.2 mg/kg and at 24 h after 650 ng/kg Administration
Each point represents the mean value of three experiments. The line shows a positive correlation.

Fig. 6. The Concentration of Each Subcellular Fraction in the Heart and Lung at 5, 20 and 120 min after 650 ng/kg i.v. Administration of \(^{3}H\)-QNB in Rats
Each bar represents the mean±S.E.M. of three experiments. ■, 5 min; □, 20 min; □, 120 min.

Fig. 7. The Relationship between the Protein Content and Subcellular Fraction-to-Plasma Concentration Ratio at Steady-state in Each Subcellular Fraction
Each point represents the mean±S.E.M. of three experiments. The solid line represents the linear regression.
We reported that there was a good relationship between the $K_{a}$ and drug lipophilicity. At the high dose, it was suggested that the characteristics of subcellular distribution for anticholinergic drugs were similar since the subcellular distribution of BP and TP in the brain, heart and lung corresponded well with that of QNB, the ligand of a typical muscarinic antagonist (Figs. 2, 3). The affinity of these drugs to the tissue distribution was in the following order: lung (highest), heart and brain. However, the concentration of each subcellular fraction in the brain after BP or TP i.v. administration was about 4 times higher than that for QNB. These results reflected the drug lipophilicity because the octanol–water partition coefficients of TP, BP and QNB were 1470, 678 and 40, respectively.

We tried to clarify the concentration–time courses of these drugs in the brain subcellular fractions following high dose i.v. administration (Fig. 1). The concentration–time courses in various brain regions for 4 h after BP i.v. administration exhibited decreases approximately parallel to that of plasma. The concentration–time courses of BP in the total brain in this study agreed with those previously reported (data not shown). However, there were two major patterns in the concentration–time courses of these drugs: a parallel or a slow decrease as compared to the plasma concentrations. These results conflict with those in the total brain. However, the percentage for the synaptic membrane and mitochondria, where the concentrations decreased slowly, of the total BP concentration in the brain, was less than 13%. Therefore, the concentration–time course of total brain decreased approximately parallel to that of plasma, in spite of the existence of fractions in which the concentrations decreased slowly.

We previously reported on the tissue distribution of QNB. After administering a dose of 3.2 mg/kg, the brain concentration increased rapidly within 5 min, whereas after a dose of 325 ng/kg, the concentration increased gradually up to 1 h; however, the plasma concentration was dose-independent. To compare the subcellular distribution at equilibrium of each dosage, the relationship of the percentage of brain subcellular fraction to the total concentration of QNB at 20 min for a high dose and at 24 h for a low dose was examined (Fig. 5). A significant difference between the two was found. At a high dose, the subcellular distribution involved nonspecific binding which correlated with the protein amount of each fraction, whereas at a low dose, QNB has higher affinity for P12, P3, and P4 than the other fractions. Therefore, it was found that QNB which accumulated in the fractions was associated with the appearance of the drug effect and/or drug metabolism at a low dose. We previously reported that QNB had two different affinity sites, one was a linear, reversible and non-specific binding site and the other was nonlinear, irreversible and specific. The total concentration-time course of $^3$H-QNB in the heart decreased temporally at 20 min (Fig. 6). It seems reasonable that the decrease reflects the distribution kinetics of a linear, reversible and non-specific binding site. The reason why the $^3$H-QNB concentration in the lung continued to decrease until 2 h is that the maximal binding capacity ($B_{max}$) of the lung was about 1/5 smaller than that of the heart. I would suggest that if its concentration reached the $B_{max}$, $^3$H-QNB accumulation in the lung would be the same as heart. The primary characteristics of the subcellular distribution in heart are essentially similar to those in the brain. At a high dose, the subcellular distribution was dependent on the protein amount of each fraction and decreased approximately parallel to the plasma concentration, whereas at a low dose, it accumulated for 2 h. These results suggest that the decreasing concentration–time courses of QNB at a high dose for the brain and heart reflect the protein binding in the non-specific binding sites, whereas the accumulation in these tissues at the same dose dependent on the irreversible protein binding in the specific binding sites rather than the receptor binding because the concentration–time courses in each fraction did not change with time.

However, the pattern of subcellular distribution in the lung differed clearly from that in the heart and brain. In particular, the P3 fraction was about 35% of the total concentration in the lung at 20 min after i.v. BP, TP or QNB administration, compared to about 10% in heart (Fig. 3). Moreover, in the lung at 2 h this increased to about 55%. At a low dose, the subcellular distribution of $^3$H-QNB in the lung was particularly large in P3, whereas it was small in P3 (Fig. 6). Therefore, the distribution of basic drugs in the lung is due to P3, regardless of dosage, and the contribution of P3 increases with decreasing blood concentration. This differs substantially from the case in the brain and heart.

The P3 fraction is occupied by mitochondria and lysosomes. We examined the subcellular distribution of basic drugs in rat liver, and found that lysosomes and acid granules have a high affinity for basic drugs in vivo. The two predominates, especially in the lung. Therefore, it is suggested that the high affinity for P3 in the lung is dependent on lysosomes.

In conclusion, we elucidated that although the tissue concentration–time courses of anticholinergic drugs appear to decrease simply parallel to plasma concentration, the pattern of subcellular distribution is characteristically dependent on the concentration among various tissues.

REFERENCES