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Effect of Sequence of Administration on the Pharmacokinetic Interaction between the Anticholinergic Drug Biperiden and [^{3}H]Quinuclidinyl Benzylate or [^{3}H]*N*-Methylscopolamine in Rats

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Abstract

In rats the pharmacokinetic interactions between the anticholinergic drug biperiden and [^{3}H]quinuclidinyl benzylate ([^{3}H]QNB) or [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) is affected by the sequence in which the drugs are administered.

Drug concentrations in various tissues were determined after intravenous administration of [^{3}H]QNB or [^{3}H]NMS (325 ng kg^{-1}). Biperiden (6·4 mg kg^{-1}) was administered either 5 min before, concomitantly with or 20 min after injection of [^{3}H]QNB or [^{3}H]NMS. When biperiden was administered concomitantly with or before [^{3}H]QNB, distribution of [^{3}H]QNB among the regions of the brain and other tissues was reduced; at 4 h the ratio of the distribution of [^{3}H]QNB for experimental animals to that for control animals ranged from 0·15 to 0·9. When biperiden was administered after [^{3}H]QNB, the distribution of [^{3}H]QNB in the brain and other tissues was significantly higher than for the other two treatments ($P < 0·01$). However, for [^{3}H]NMS the sequence of administration had no effect on the distribution of the drug in the brain and other tissues except for the kidney. In-vitro, in crude synaptosomal membranes, the amount of [^{3}H]QNB at 2 h relative to the control concentration at equilibrium was 87% when biperiden was added before [^{3}H]QNB and 56% when biperiden was added after [^{3}H]QNB. In both instances the concentration of [^{3}H]NMS reached equilibrium within 30 min.

These findings suggest that the difference between the rate constant of association and dissociation at the possible site of action gives rise to the effect of the sequence of administration on the pharmacokinetic interaction.

It is well known that the main causes of pharmacokinetic interactions are gastrointestinal absorption, protein binding and metabolic enzymes. In contrast, although plural receptor antagonists are often used simultaneously in drug therapy for central nervous system diseases, little is known about the changes in pharmacokinetic disposition that occur as a result of competition for receptors. Several studies have been performed on the inhibitory effects of anticholinergic drugs in-vitro using [^{3}H]quinuclidinyl benzylate ([^{3}H]QNB) and [^{3}H]*N*-methylscopolamine ([^{3}H]NMS). However, in-vivo studies are needed to clarify how the distribution disposition of a drug is affected by its interactions with receptors.

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[^{3}H]QNB and [^{3}H]NMS are typical ligands which non-selectively and specifically bind to muscarinic acetylcholine receptors. These ligands are used to measure receptor occupancy in regions of the rat brain (Yamamura & Snyder 1974; Westland et al 1981; Esam et al 1986). Although the antimuscarinic potencies of various anti cholinergic drugs have been studied by inhibiting [^{3}H]QNB binding in-vitro (Watanabe et al 1983), Yamamura et al (1974) reported that the number of [^{3}H]QNB binding sites (B_{max}) differed in-vitro and in-vivo, with the in-vitro B_{max} being only 25–30%

that calculated from in-vivo experiments. Therefore, in-vivo rather than in-vitro studies are needed to elucidate the pharmacokinetic interactions involved in the inhibition of receptor-binding.

We recently examined the disposition kinetics of [^{3}H]QNB using a physiologically based pharmacokinetic model and pharmacokinetics including receptor binding (Ishizaki et al 1992). Biperiden, clinically used as an antagonistic drug, inhibits activation by binding to the muscarinic acetylcholine receptor and has selectivity for the M_1 binding site (Larson et al 1991). We have been studying the pharmacokinetic disposition of biperiden, including its distribution in rat brain (Yokogawa et al 1990, 1992).

Basing our results solely on the pharmacokinetic disposition of QNB and biperiden, this study investigated the effect of drug interaction on the distribution disposition of receptor-binding sites using biperiden, [^{3}H]QNB and [^{3}H]NMS.

Materials and Methods

Materials

[^{3}H]QNB (41·6 Ci mmol^{-1}) and [^{3}H]NMS (83 Ci mmol^{-1}) were purchased from Amersham International (Bucks, UK). Biperiden (Dainippon, Osaka, Japan) was used as supplied. Other chemicals were of reagent grade and were used without purification. The drugs were dissolved in 50 mM tris-HCl buffer (pH 7·4).

In-vivo animal experiments

Male Wistar rats (250 ± 22 *g*, mean ± s.d.; Sankyo Laboratory Animal, Toyama, Japan) were used randomly in this study. The experiments were performed essentially as described elsewhere (Yokogawa et al 1990). On the basis of previous work the dose of [^{3}H]QNB or [^{3}H]NMS chosen for this study was 325 ng kg^{-1} (Ishizaki et al 1992). A 0·24-mL sample of [^{3}H]QNB or [^{3}H]NMS solution was injected into rats via a femoral vein with or without 6·4 mg kg^{-1} biperiden. The rats were killed at designated times after [^{3}H]QNB or [^{3}H]NMS dosing for sampling of tissues of the brain, heart, lung, kidney and muscle. The brains were dissected within 3 min into six regions (frontal cortex, basal ganglia, thalamus, pons + medulla oblongata, hippocampus and cerebellum) according to the method of Gispen et al (1972).

Preparation of rat synaptosomal membrane

Rat brain was homogenized in 10% (w/w) ice-cold 0·32 M sucrose solution according to the method of Watanabe et al (1983). The homogenate was centrifuged for 10 min at 1000 *g* at 4°C. The supernatant was re-centrifuged for 20 min at 27 000 *g* and the intermediate pellets were washed twice with ice-cold 0·32 M sucrose solution. The final pellets (synaptosomal membrane fraction) were re-suspended in 50 mM tris-HCl buffer (pH 7·4) to a protein concentration of 750 μg mL^{-1} and stored at −80°C until used. The homogenate was diluted with 50 mM tris-HCl buffer (pH 7·4) before the experiment and used within 24 h. Protein concentration was determined by use of a commercial Kit (Protein Assay Kit, Bio-Rad Laboratories, Osaka, Japan).

Binding studies of [^{3}H]QNB and [^{3}H]NMS in-vitro

In-vitro binding studies of [^{3}H]QNB or [^{3}H]NMS were performed according to the method of Watanabe et al (1983), with minor modification. Briefly, 0·4 mL (300 μg protein) of synaptosomal membrane was incubated with 0·4 mL [^{3}H]QNB or [^{3}H]NMS (10^{-10} M final concentration) and with 0·4 mL biperiden (final concentration $10^{-4}-10^{-11}$ M) or 50 mM tris-HCl buffer (pH 7·4). The final total volume was 1·2 mL. Incubation was continued for between 0 and 360 Min at 37°C.

Assay for [^{3}H]QNB and [^{3}H]NMS

The concentrations of [^{3}H]QNB and [^{3}H]NMS in various regions of the brain and in other tissues were determined by a method described elsewhere (Ishizaki et al 1992) with slight modifications. Briefly, the radioactivity in tissue samples was determined directly after combustion with an oxidiser (Aloka ASC-113). The binding reaction was terminated by filtering the assay mixture through a GF/G glass-fibre filter (Whatman, Clifton, NJ) under vacuum, and each filter was rinsed twice with 50 mM tris-HCl buffer (pH 7·4). Filters were placed in vials with 16 mL scintillation fluid (ACS-II; Amersham, Arlington Heights, IL), maintained at 20°C for 12 h and the radioactivity was then assayed by liquid scintillation spectrometry (LSC-3600 liquid scintillation counter; Aloka, Japan).

Data analysis

The area under the concentration-time curve (AUC) for various regions of the brain and for other tissues was estimated using the trapezoidal rule (Yamaoka et al 1981). Student's *t*-test was used to compare the unpaired means of two sets of data. A P value < 0.05 was taken as indicative of a significant difference between sets of data.

Results

Figures 1 and 2 illustrate the concentration–time profiles of [^{3}H]QNB in various regions of the brain and other tissues for 10 h after an intravenous dose

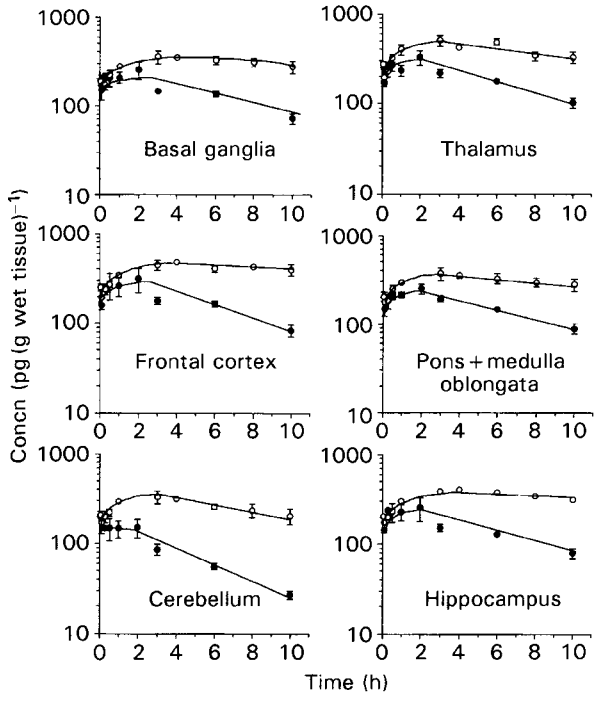

Figure 1. Concentration–time courses of [^{3}H]QNB (325 ng kg^{-1}) in the different regions of the rat brain without (○, control) and with (●) simultaneous intravenous administration of biperiden (6·4 mg kg^{-1}). Each point is the mean ± s.e.m. of results from 3 or 4 rats.

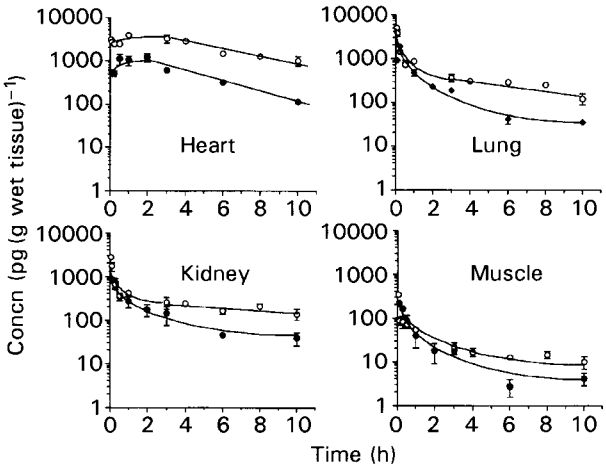

Figure 2. Concentration–time courses of [^{3}H]QNB (325 ng kg^{-1}) in rat tissues without (○, control) and with (●) simultaneous intravenous administration of biperiden (6·4 mg kg^{-1}). Each point is the mean ± s.e.m. of results from 3 or 4 rats.

of 325 ng kg^{-1} in rats. After administration of [^{3}H]QNB alone concentrations in the brain increased gradually up to 3 h, and decreased thereafter, the elimination rate-constants ranging from 0·01 to 0·068 h^{-1}. The ranking order of the decreasing rates was cerebellum > thalamus > pons + medulla oblongata > basal ganglia > hippocampus > frontal cortex. Though the maximum concentration in lung, kidney and muscle was reached within 5 min, in heart it took 1–2 h. The elimination rate-constants in heart, lung, kidney and muscle were 0·14, 0·045, 0·035 and 0·031 h^{-1}, respectively. Elimination was more rapid in the heart than in other tissues.

When biperiden (6·4 mg kg^{-1}) was administered concomitantly with [^{3}H]QNB the concentrations of [^{3}H]QNB in all regions of the brain except the cerebellum were found not to be inhibited by biperiden during the first 30 min. However, after 60 min these concentrations were reduced to approximately 75% those for control to which [^{3}H]QNB alone was administered. The concentration in the cerebellum diminished immediately on administration of biperiden, and the concentration decreased to approximately 50% of the control value 60 min after intravenous injection. Ten hours after administration the concentrations in the regions of the brain were 13·4–27% the control values. The concentration of [^{3}H]QNB in heart decreased immediately compared with the control after intravenous dosing whereas those in lung, kidney and muscle did not change for 30 min and later gradually decreased.

Table 1 lists the AUC values of [^{3}H]QNB from 0 to 10 h for various regions of the brain and for other tissues with or without administration of biperiden. When biperiden was administered simultaneously, AUC values in all regions of the brain and in other tissues except muscle were significantly smaller than those in control animals ($P<0·01$). In both heart and cerebellum the AUC was reduced by more than 70% by administration of biperiden; this was more than in other regions of the brain and in other tissues.

Table 2 shows the ratio of the [^{3}H]QNB concentration to that in the control for various regions of the brain and for other tissues on administration of biperiden (3·2, 19·2, 96, 960, 9600 μg kg^{-1}) 5 min before [^{3}H]QNB. These concentrations were determined 4 h after [^{3}H]QNB injection. Although the binding of QNB was not inhibited immediately after administration of biperiden (3·2 μg kg^{-1}), the ratio to the control value for every region of the brain and for other tissue tended to decrease gradually with increasing dose of biperiden. For biperiden at 9·6 mg kg^{-1}, the ratio varied from 0·15 (heart) to 0·67 (muscle).

The sequential effects of administration of biperiden on the distribution of [^{3}H]QNB or [^{3}H]NMS were studied. Biperiden was administered either 5 min before, concomitantly with or 20 min after injection of [^{3}H]QNB. Table 3 shows the reduction relative to the control of the concentration of [^{3}H]QNB in various regions of the

Table 1. Effect of biperiden on the area under the plasma concentration–time curve of [^{3}H]quinuclidinyl benzylate from 0 to 10 h.

Brain region or tissue	Control* (ng min mL^{-1} kg^{-1})	With biperiden† (ng min mL^{-1} kg^{-1})	Decrease‡ (%)
Frontal cortex	4·03 ± 0·19	1·78 ± 0·16	55·8
Basal ganglia	3·08 ± 0·2	1·46 ± 0·12	52·6
Thalamus	4·09 ± 0·24	1·94 ± 0·12	52·6
Pons plus medulla oblongata	3·14 ± 0·19	1·63 ± 0·11	48·1
Hippocampus	3·5 ± 0·14	1·49 ± 0·12	57·4
Cerebellum	2·67 ± 0·17	0·79 ± 0·07	70·4
Heart	21·9 ± 1·66	5·05 ± 0·46	76·9
Lung	4·4 ± 0·22	1·95 ± 0·12	55·7
Kidney	2·66 ± 0·21	1·25 ± 0·22	53·0
Muscle	0·25 ± 0·02	0·19 ± 0·03	23·1

Values are means ± s.d. *Area under the plasma concentration–time curve of [^{3}H]quinuclidinyl benzylate after intravenous administration of [^{3}H]quinuclidinyl benzylate (325 ng kg^{-1}) alone. †Area under the plasma concentration–time curve of [^{3}H]quinuclidinyl benzylate after concomitant administration of [^{3}H]quinuclidinyl benzylate (325 ng kg^{-1}) and biperiden (6·4 mg kg^{-1}). ‡The percentage decrease in the area under the plasma concentration–time curve as a result of the simultaneous administration of biperiden.

Table 2. Effect of different concentrations of biperiden on the concentration of [^{3}H]quinuclidinyl benzylate in various regions of the rat brain and in other tissues.

Brain region or tissue	Ratio for biperiden concentration (μg kg^{-1})				
	3·2	19·2	96	960	9600
Frontal cortex	1·01 ± 0·031	0·937 ± 0·026	0·82 ± 0·047	0·546 ± 0·013	0·223 ± 0·03
Basal ganglia	0·975 ± 0·05	0·93 ± 0·024	0·898 ± 0·057	0·641 ± 0·037	0·365 ± 0·03
Thalamus	0·985 ± 0·092	0·918 ± 0·048	0·855 ± 0·051	0·639 ± 0·041	0·397 ± 0·041
Pons plus medulla oblongata	0·998 ± 0·093	0·859 ± 0·027	0·788 ± 0·064	0·664 ± 0·03	0·421 ± 0·045
Hippocampus	0·934 ± 0·083	0·837 ± 0·024	0·781 ± 0·061	0·514 ± 0·033	0·277 ± 0·027
Cerebellum	0·954 ± 0·075	0·929 ± 0·036	0·795 ± 0·073	0·524 ± 0·04	0·212 ± 0·015
Heart	1·0 ± 0·087	0·891 ± 0·044	0·815 ± 0·111	0·573 ± 0·028	0·151 ± 0·007
Lung	1·156 ± 0·144	0·767 ± 0·1	0·614 ± 0·055	0·449 ± 0·052	0·324 ± 0·051
Kidney	0·909 ± 0·073	0·598 ± 0·046	0·449 ± 0·047	0·271 ± 0·01	0·358 ± 0·054
Muscle	0·961 ± 0·102	0·739 ± 0·114	0·587 ± 0·034	0·633 ± 0·079	0·674 ± 0·052

Values are ratios of the concentration of [^{3}H]quinuclidinyl benzylate in experimental tissue to that in control tissue (no biperiden) 4 h after intravenous injection of biperiden and, 5 min later, [^{3}H]quinuclidinyl benzylate (325 ng kg^{-1}). Values are the means ± s.e.m. of results from 3–6 rats.

brain and in other tissues when biperiden was co-administered 4 h after intravenous [^{3}H]QNB. When biperiden was administered concomitantly with or before [^{3}H]QNB, the values ranged from 0·15 to 0·9 among the regions of the brain and in other tissues, with no difference between the two treatments. However, when biperiden was administered after [^{3}H]QNB, the distribution of [^{3}H]QNB in tissues was significantly ($P < 0·01$) higher than for the other two treatments.

Table 4 shows the ratio of the [^{3}H]NMS concentration to that in the control for regions of the brain and for other tissues on co-administration of biperiden 4 h after intravenous [^{3}H]NMS. There were no significant differences between the results obtained when biperiden was administered before or after intravenous administration of [^{3}H]NMS. In kidney, however, reduction in the amount of [^{3}H]-NMS was significantly ($P < 0·01$) different from that in the control, behaviour similar to that of [^{3}H]QNB.

Next, to examine the cause of the pharmacokinetic interactions, an in-vitro binding study was performed using rat brain crude synaptosomal membranes. Figure 3 illustrates the inhibitory effect of different concentrations of biperiden on binding of [^{3}H]QNB (10^{-10} M) to the synaptosomal membrane at 37°C. Biperiden was added either 5 min before or 20 min after addition of [^{3}H]QNB. When biperiden (10^{-5} M) was added before [^{3}H]QNB the binding was immediately completely blocked. On the other hand, when 10^{-5} M biperiden was added after [^{3}H]QNB, the time course of [^{3}H]QNB concentration decreased slowly from maximum binding, 460 fmol (mg protein)$^{-1}$, and reached approximately 26% of maximum binding at 360 min. When biperiden (10^{-11} M) was added before [^{3}H]QNB, the binding of [^{3}H]QNB increased rapidly and reached approximately 90%

Table 3. Effect of the time of administration of biperiden on the concentration of [^{3}H]quinuclidinyl benzylate in various regions of the rat brain and in other tissues.

Brain region or tissue	Biperiden then [^{3}H]quinuclidinyl benzylate	Biperiden and [^{3}H]quinuclidinylbenzylate simultaneously	[^{3}H]Quinuclidinyl benzylate then biperiden
Frontal cortex	0·704 ± 0·03	0·647 ± 0·011	0·316 ± 0·052*
Basal ganglia	0·635 ± 0·03	0·603 ± 0·009	0·258 ± 0·036*
Thalamus	0·603 ± 0·041	0·54 ± 0·001	0·286 ± 0·066*
Pons plus medulla oblongata	0·579 ± 0·045	0·525 ± 0·017	0·267 ± 0·022*
Hippocampus	0·723 ± 0·027	0·691 ± 0·009	0·326 ± 0·056*
Cerebellum	0·788 ± 0·015	0·806 ± 0·003	0·558 ± 0·045*
Heart	0·849 ± 0·007	0·9 ± 0·004	0·636 ± 0·043*
Lung	0·676 ± 0·051	0·744 ± 0·04	0·317 ± 0·067*
Kidney	0·642 ± 0·054	0·715 ± 0·029	0·156 ± 0·063*
Muscle	0·326 ± 0·052	0·376 ± 0·066	0·145 ± 0·025†

Biperiden (9·6 mg kg^{-1}) was administered to the rats 5 min before, concomitantly with, or 20 min after intravenous injection of [^{3}H]quinuclidinyl benzylate (325 ng kg^{-1}). Values are ratios of the concentration of [^{3}H]quinuclidinyl benzylate in experimental tissue to that in control tissue (no biperiden) 4 h after injection and are the means ± s.e.m. of results from 3–6 rats. †$P < 0·05$, *$P < 0·001$, significantly different from the result obtained when biperiden was administered before [^{3}H]quinuclidinyl benzylate.

Table 4. Effect of the time of administration of biperiden on the concentration of [^{3}H]*N*-methylscopolamine in various regions of the rat brain and in other tissues.

Brain region or tissue	Biperiden then [^{3}H]*N*-methylscopolamine	[^{3}H]*N*-methylscopolamine then biperiden
Frontal cortex	0·378 ± 0·053	0·276 ± 0·064
Basal ganglia	0·185 ± 0·072	0·225 ± 0·107
Thalamus	0·342 ± 0·171	0·33 ± 0·098
Pons plus medulla oblongata	0·257 ± 0·107	0·132 ± 0·096
Hippocampus	0·414 ± 0·075	0·316 ± 0·093
Cerebellum	0·351 ± 0·051	0·295 ± 0·041
Heart	0·896 ± 0·007	0·911 ± 0·007
Lung	0·708 ± 0·025	0·771 ± 0·016
Kidney	0·675 ± 0·016	0·282 ± 0·053*
Muscle	0·210 ± 0·057	0·169 ± 0·106

Biperiden (9·6 mg kg^{-1}) was administered to the rats either 5 min before or 20 min after intravenous injection of [^{3}H]*N*-methylscopolamine (325 ng kg^{-1}). Values are ratios of the concentration of [^{3}H]*N*-methylscopolamine in experimental tissue to that in control tissue (no biperiden) 4 h after injection and are the means ± s.e.m. of results from 3–6 rats. *$P < 0·001$, significantly different from the result obtained when biperiden was administered before [^{3}H]N-methylscopolamine.

of the maximum within 20 min. On the other hand, when biperiden (10^{-11} M) was added after [^{3}H]QNB, the binding of [^{3}H]QNB was only slightly inhibited from the beginning. The concentration of biperiden resulting in 50% inhibition (IC50 value) of binding of [^{3}H]QNB (10^{-10} M), obtained from the competitive experiment at 37°C for 6 h, was 5×10^{-8} M (data not shown). When biperiden (5×10^{-8} M) was added concomitantly, the maximum binding of [^{3}H]QNB at equilibrium was 230 fmol (mg protein)$^{-1}$. However, at 120 min the binding of [^{3}H]QNB was only approximately 87% of the maximum when biperiden was added before [^{3}H]QNB but only approximately 56% of the maximum when biperiden was added after [^{3}H]QNB. Both differences were significant.

Similarly, Figure 4 illustrates the inhibitory effect of different concentrations of biperiden on binding of [^{3}H]NMS (10^{-10} M) to the synaptosomal membrane at 37°C. The IC50 value of biperiden for [^{3}H]NMS (10^{-10} M) binding was 10^{-8} M (data not shown). [^{3}H]NMS binding reached equilibrium within 30 min when biperiden (10^{-8} M) was added either before or after [^{3}H]QNB.

Table 5 lists the binding parameters of [^{3}H]QNB and [^{3}H]NMS obtained by competitive inhibition studies (Figures 3 and 4). These parameters were estimated according to Yang et al (1986). The association rate-constant (k_1) of [^{3}H]QNB was approximately equal to that of [^{3}H]NMS, but the dissociation rate constant (k_{-1}) of [^{3}H]NMS was 6·5 times larger than that of [^{3}H]QNB. The equilibrium dissociation constant (K_d) of [^{3}H]NMS calculated from the rate constant ratio k_{-1}/k_1, was approximately five times larger than that of [^{3}H]QNB.

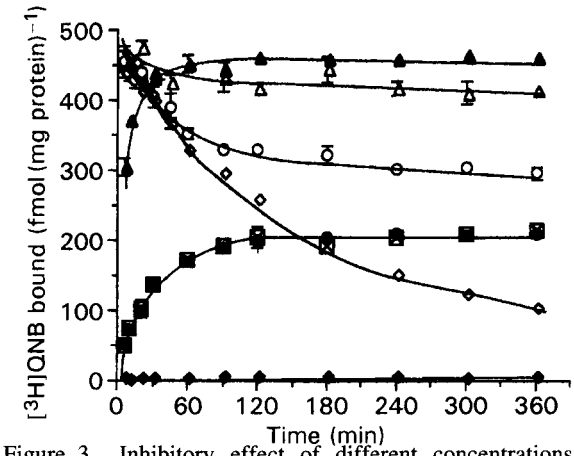

Figure 3. Inhibitory effect of different concentrations of biperiden (△, ▲, 10^{-11} M; ○, ●, 5×10^{-8} M; ◇, ◆, 10^{-5} M) on the time-courses of the in-vitro binding of [^{3}H]QNB (10^{-10} M) with synaptosomal membrane at 37°C. Three different sets of conditions were used: open symbols indicate that the membrane was preincubated with [^{3}H]QNB for 1 h before addition of biperiden; closed symbols indicate that the membrane was preincubated with biperiden for 1 h before addition of [^{3}H]QNB; ⊠ indicates that the membrane was incubated concomitantly with [^{3}H]QNB (10^{-10} M) and biperiden (5×10^{-8} M). Each point is the mean ± s.e.m. of results from 3 rats.

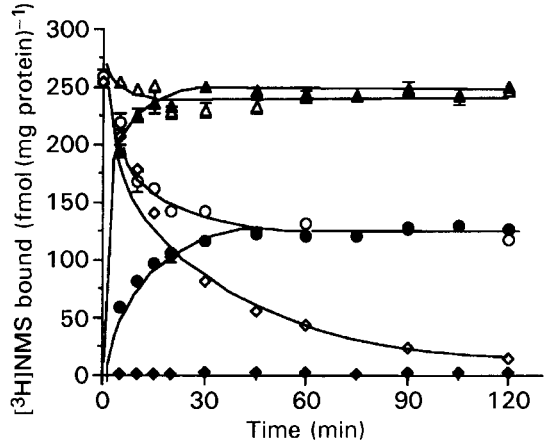

Figure 4. Inhibitory effect of different concentrations of biperiden (△, ▲, 10^{-11} M; ○, ●, 10^{-8} M; ◇, ◆, 10^{-4} M) on the time-courses of the in-vitro binding of [^{3}H]NMS (10^{-10} M) with synaptosomal membrane at 37°C. Two different sets of conditions were used: open symbols indicate that the membrane was preincubated with [^{3}H]NMS for 1 h before addition of biperiden, closed symbols indicate that the membrane was preincubated with biperiden for 1 h before addition of [^{3}H]NMS. Each point is the mean ± s.e.m. of results from 3 rats.

Discussion

This study has discovered that the pharmacokinetic interaction between [^{3}H]QNB and biperiden at the same receptor binding site is affected by the sequence in which the drugs are administered.

Previously, we reported that the distribution of QNB at high dose (3·2 mg kg^{-1}) is non-specific and that its binding in different regions of the brain and in other tissues is linear, whereas its distribution at low dose (325 ng kg^{-1}) is irreversible and binding is non-linear (Ishizaki et al 1992). Therefore, to observe pharmacokinetic interaction at the same receptor binding site it is necessary to use low doses of [^{3}H]QNB. When biperiden (6·4 mg kg^{-1}) was administered concomitantly with a low dose of [^{3}H]QNB, after 10 h the concentration of [^{3}H]QNB in every region of the brain and in other tissues was significantly lower than that in the control. However, the inhibitory effects of biperiden occurred more rapidly in cerebellum and heart than in the other tissues. The percentage reduction in the AUC for these tissues was significantly larger than that for other regions of the brain and for other tissues. The reason for this is possibly differences between the rates of distribution in, and the affinities of, the different regions of the brain and other tissues. However, we have previously reported that the highly lipophilic biperiden was rapidly distributed to all tissues after an intravenous dose (Yokogawa et al 1992). In that work the affinity of cerebellum was the smallest of the various regions of the brain and the affinities of the brain and heart were approximately one ninth that of the lung. Because there was no agreement between the affinity of a tissue for biperiden and the percentage reduction of the AUC of [^{3}H]QNB by biperiden in that tissue, the difference in the [^{3}H]QNB concentration might depend on the distribution kinetics of [^{3}H]QNB rather than of biperiden.

Watson et al (1983) demonstrated that the binding sites for M_1 muscarinic subtype in cerebellum and heart are only 10·4 and 3·1%, respectively, of the total [^{3}H]QNB-binding sites, most of which were of the M_2 subtype. Waelbroeck et al (1991) reported that although the association of [^{3}H]QNB to binding sites did not differ significantly among the various muscarinic subtypes, the dissociation of [^{3}H]QNB from the M_2 subtype was more rapid than that from the M_1 subtype. Therefore, it was thought that the inhibitory effect by biperiden appeared more rapidly in the cerebellum and heart, areas rich in the M_2 subtype, than in the other regions of the brain and other tissues. On the other hand, it has been reported that the affinity of biperiden for muscarinic receptors was 5–10 times higher for the M_1 subtype than for the M_2 subtype (Larson et al 1991). However, the influence of biperiden on [^{3}H]QNB seemed to be more predominant in the cerebellum and the heart than in the other tissues. The inhibitory effects were caused by the dissociation of [^{3}H]QNB from the binding site rather than by the selectivity of the biperiden receptor subtype.

Table 5. Binding parameters of [^{3}H]quinuclidinyl benzylate and [^{3}H]*N*-methylscopolamine.

Parameter	[^{3}H]Quinuclidinyl benzylate	[^{3}H]*N*-Methylscopolamine
Rate constant for association ($\times 10^8$ M^{-1} min^{-1})	1·36 ± 0·06	1·84 ± 0·3
Rate constant for dissociation (min^{-1})	0·00406 ± 0·00008	0·0264 ± 0·0018
Equilibrium dissociation constant (pM)	29·9	143·5
Maximum number of binding sites (pmol (mg protein)$^{-1}$)	2·12	1·31
Equilibrium dissociation constant of competing ligand (nM)	10·2 ± 1·0	10·8 ± 3·6

Values are means ± s.d.

The tissue concentration–time profiles of [^{3}H]QNB in lung, kidney and muscle during the first 30 min were unaffected by administration of biperiden. This finding explains the reduction in non-specific binding because the maximum binding capacities (B_{max}) of lung, kidney and muscle were smaller than those of the regions of the brain and of the heart (Ishizaki et al 1992). The inhibitory effect on [^{3}H]QNB of increasing the dose of biperiden was examined (Table 2). The dose of biperiden required for a 50% reduction of [^{3}H]QNB (IC50) was 1090–3450 μg kg^{-1} in the regions of the brain and 97, 650 and 980 μg kg^{-1} in the kidney, lung and heart, respectively. This suggested that the concentrations of [^{3}H]QNB in these tissues were influenced by lower doses of biperiden than those in the regions of the brain.

Moreover, we found that this drug interaction depends on the sequence of administration. The inhibitory effect of biperiden on the distribution of [^{3}H]QNB was observed whatever the sequence of administration. However, its effect when biperiden was administered concomitantly or before [^{3}H]QNB was significantly greater than when biperiden was administered after [^{3}H]QNB. The effect of the sequence of administration was characteristically different in the heart and cerebellum, which are rich in the M_2 subtype, from that in the other regions of the brain and other tissues. On the other hand, the sequence of administration of biperiden and [^{3}H]NMS had no effect other than in the kidney.

To elucidate the reason for this we conducted in-vitro binding studies using rat brain synaptosomal membranes. [^{3}H]QNB binding reached equilibrium more rapidly when biperiden was added before rather than after [^{3}H]QNB (Figure 3). Concomitant addition resulted in a pattern similar to when biperiden was added before [^{3}H]QNB. However, in both experiments the concentration of [^{3}H]NMS reached equilibrium at the same time—within 30 min (Figure 4). The values of K_d and B_{max} for the binding characteristics of [^{3}H]QNB (Table 5) were similar to those of the muscarinic cholinoreceptors described previously for brain (Yamamura & Snyder 1974). The affinity of [^{3}H]QNB was approximately five times higher than that of [^{3}H]NMS. However, the k_1 of [^{3}H]QNB was approximately equal to that of [^{3}H]NMS, whereas k_{-1} of [^{3}H]QNB was approximately one sixth that of [^{3}H]NMS. There were no marked differences between the IC50 values of biperiden for [^{3}H]QNB and [^{3}H]NMS, 5×10^{-8} M and 10^{-8} M, respectively. These results imply that the cause of the sequential effect of administration of biperiden on [^{3}H]QNB is the difference between the rates of association and dissociation of [^{3}H]QNB in the presence of biperiden.

It remains unclear why the effect of the sequence of administration for [^{3}H]NMS in the kidney differed from that in the brain and other tissues, and why the effect for [^{3}H]QNB was highest in the kidney. Sandmann et al (1991) reported that the muscarinic M_3 receptor subtype is present in the embryonic kidney in man; this suggests that the kidney contains a muscarinic receptor subtype for which dissociation is smaller than for the M_1 and M_2 subtypes.

In conclusion, this effect of the sequence of administration is a result of different rates of association and dissociation of [^{3}H]QNB rather than the strength of the affinity of biperiden for the site of action. The magnitude of the effect differed among the tissues, suggesting that the cause is the different distributions of various subtypes of muscarinic receptors. Thus, although [^{3}H]QNB and [^{3}H]NMS are not used clinically, it is essential to consider the effects of their sequence of administration on pharmacokinetic interactions resulting from receptor competition.

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