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PROPIVERINE AND ITS N-OXIDE METABOLITE BIND SIGNIFICANTLY TO L-TYPE CALCIUM CHANNEL ANTAGONIST RECEPTORS IN RAT BLADDER

Hypothesis / aims of study

Propiverine (1-methyl-4-piperidyl diphenylpropoxyacetate) is commonly used as anticholinergic agent for the treatment of patients with overactive bladder [1]. In muscle strips of guinea-pig urinary bladder, propiverine has been shown to inhibit contractions induced by acetylcholine and KCl [2]. Further, this drug exerted competitive anticholinergic and calcium-antagonistic effects in the isolated urinary bladder of rats, dogs and human [3]. Propiverine is metabolized in the liver to form active metabolites, 1-methyl-4-piperidyl diphenylpropoxyacetate N-oxide (P-4(N→O)) and 1-methyl-4-piperidyl benzilate N-oxide (DPr-P-4(N→O)). In *in vitro* and *in vivo* pharmacological studies, it was previously shown that these metabolites exerted anticholinergic and calcium antagonistic effects in the urinary bladder of rats and guinea-pigs [3]. Taken together, it is plausible that propiverine and its metabolites may inhibit the calcium influx through the blockade of calcium channels in the urinary smooth muscle. However, the calcium channel antagonistic activities of propiverine and its metabolites have not been investigated directly by radioligand binding assays.

It has been shown that voltage dependent L-type calcium channel antagonists of diverse chemical structures interact in an allosteric manner with the calcium ion-channel protein. These antagonists are chemically heterogenous in natures and have been classified broadly into the following three groups: dihydropyridines, phenylalkylamines and benzothiazepines. The aim of the current study was to clarify the binding activities by propiverine and its metabolites of L-type calcium channel antagonist receptors in the bladder and brain of rats.

Study design, materials and methods

The radioligand binding assays for L-type calcium channel antagonist receptors were performed using (+)-[^{3}H]PN 200-110, [^{3}H]verapamil and [^{3}H]diltiazem. Muscarinic receptors in tissue homogenates were measured by radioreceptor binding assay with [N-methyl-^{3}H]scopolamine ([^{3}H]NMS) as a radioligand, and binding parameters of apparent dissociation constant (Kd) and maximal number of binding sites (Bmax) for each radioligand were estimated by Scatchard analysis. The ability of anticholinergic agents and metabolites to inhibit specific radioligand binding was estimated from IC_{50} values, namely the molar concentrations of unlabeled drugs necessary to displace 50% of specific radioligand binding. Ki was calculated by using the equation, $Ki=IC_{50}/(1+L/Kd)$, where L is the concentration of radioligand. The Ki values express the potency of anticholinergic agents in competing for radioligand binding sites in human tissues.

Results

Propiverine and P-4(N→O) inhibited specific (+)-[^{3}H]PN 200-110 binding in the rat bladder in a concentration-dependent manner. Compared with that for propiverine, the Ki value for P-4(N→O) in the bladder was significantly greater (Table 1). Scatchard analysis has revealed that propiverine increased significantly Kd values for bladder (+)-[^{3}H]PN 200-110 binding. DPr-P-4(N→O) had little inhibitory effects on the bladder (+)-[^{3}H]PN 200-110 binding. Propiverine and its metabolites also inhibited specific [^{3}H]NMS binding in the rat bladder. The ratios of Ki values for (+)-[^{3}H]PN 200-110 to [^{3}H]NMS were relatively small for propiverine and P-4(N→O) (Table 1). Propiverine and P-4(N→O) inhibited specific binding of (+)-[^{3}H]PN 200-110, [^{3}H]diltiazem and [^{3}H]verapamil in the rat cerebral cortex in a concentration-dependent manner. The Ki values of propiverine and P-4(N→O) for [^{3}H]diltiazem were significantly smaller than those for (+)-[^{3}H]PN 200-110 and [^{3}H]verapamil (Table 2). Further, their Ki values for [^{3}H]verapamil were significantly smaller than those for (+)-[^{3}H]PN 200-110. The Ki values of propiverine for each radioligand in the cerebral cortex were significantly ($P<0.05$) smaller than those of P-4(N→O).

Table 1. Competitive inhibition by propiverine, P-4(N→O) and DPr-P-4(N→O) of specific binding of (+)-[^{3}H]PN 200-110 and [^{3}H]NMS in the rat bladder

	Ki values (μM)		Ki of (+)-[^{3}H]PN 200-110 / Ki of [^{3}H]NMS
	(+)-[^{3}H]PN 200-110	[^{3}H]NMS	
Propiverine	18.4 ± 2.7	0.30 ± 0.04	61
P-4(N→O)	159 ± 14	6.51 ± 0.67	24
DPr-P-4(N→O)	> 1000	0.21 ± 0.01	

Values are mean ± S.E. of 3 to 4 rats.

Table 2. Competitive inhibition by propiverine and P-4(N→O) of specific binding of (+)-[^{3}H]PN 200-110, [^{3}H]diltiazem and [^{3}H]verapamil in the rat cerebral cortex

	Ki values (μM)		
	(+)-[^{3}H]PN 200-110	[^{3}H]Diltiazem	[^{3}H]Verapamil
Propiverine	43.5 ± 9.9	1.81 ± 0.39**	11.6 ± 2.5**††
P-4(N→O)	363 ± 42	40.5 ± 4.4**	112 ± 18**††

Values are mean ± S.E. of 4 to 6 rats. Asterisks show a significant difference from the (+)-[^{3}H]PN 200-110 values, **$P < 0.01$. Daggers show a significant difference from the [^{3}H]diltiazem values, ††$P < 0.01$.

Interpretation of results
The major findings of this study are that 1) propiverine and its N-oxide metabolite, P-4(N→O) binds significantly to L-type calcium channel antagonist receptors in the bladder and 2) these agents exerts higher affinity to benzothiazepine site than to dihydropyridine site and phenylalkylamine site on L-type calcium channels.

Concluding message
Propiverine and its N-oxide metabolite (P-4(N→O)) exert a significant binding activity of L-type calcium channel antagonist receptors in the bladder and these effects may be pharmacologically relevant in reducing the contractility of bladder detrusor muscle in patients with overactive bladder following oral administration of propiverine.

References

[1] Europ Urol (2000) 37; 702-708.
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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Guideline of the Experimental Animal Ethical Committee of the University of Shizuoka