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Effect of a phenyl group in quaternary ammonium compounds on thiamine uptake in isolated rat hepatocytes

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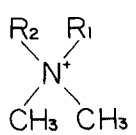
The inhibitory effect of a phenyl group in quaternary ammonium compounds on thiamine uptake in isolated rat hepatocytes was investigated. The phenyltrimethylammonium ion was a more potent inhibitor than the tetramethylammonium ion, while the dibenzyldimethylammonium ion was the most potent inhibitor of thiamine uptake among those compounds examined. A kinetic study showed that this compound was a competitive inhibitor. The cetyltrimethylammonium ion was a less effective inhibitor than the benzyltrimethylammonium ion, and the palmitoylcholine ion was a weak inhibitor. These results indicate that the lipophilicity of a quaternary ammonium compound is not always correlated with its affinity for thiamine-carrier binding, but the presence of a phenyl group plays a significant role in affinity. The inhibitory effect of the series of $(CH_3)_3N^+(CH_2)_nC_6H_5$ ($n = 0$–6) compounds on thiamine uptake in isolated rat hepatocytes was studied. The maximal inhibitory activity occurred at $n = 5$. These results suggest that the phenyl group in a quaternary ammonium compound has a specific interaction with the thiamine-binding site in rat liver plasma membrane.

Previous studies [1–4] have demonstrated that thiamine is accumulated by an active, Na^+-dependent process in isolated rat hepatocytes. In our previous papers [3–5], we have reported on the effect of quaternary ammonium compounds on thiamine uptake in isolated rat hepatocytes and have shown that the quaternary nitrogen atom is involved in the binding of these compounds to the thiamine carrier [4]. Betaine and carnitine, which have a negative charged group in their molecules, do not inhibit hepatocyte thiamine uptake, just as thiamine monophosphate and pyrophosphate do not inhibit thiamine uptake. On the other hand, choline, acetylcholine and their structural analogs are competitive inhibitors of thiamine uptake [5], even though the affinities of these compounds for the thiamine-transport site differ markedly. Although the reason for the difference in the affinities of these compounds for the thiamine-transport carrier has not been delineated, some compounds containing a phenyl group in their molecules in addition to the quaternary nitrogen atom strongly inhibited thiamine uptake. Therefore, further studies on the effect of quaternary ammonium compounds on thiamine uptake by isolated rat hepatocytes were undertaken to elucidate the binding properties of quaternary ammonium compounds.

Hepatocytes were prepared from 200–300 g male Wistar rats, fed ad libitum, as previously reported [4]. More than 90% of cells isolated in this manner routinely excluded Trypan blue. The cells were preincubated for 15 min at 37°C in an atmosphere comprising 95% O_2/5% CO_2 (v/v) in

Corning centrifugation tubes (50 ml). 3 ml of cells ($3.5 \cdot 10^6$ cells/ml) was suspended in Krebs-Henseleit medium which contained dialyzed bovine serum albumin (25 mg/ml), streptomycin (100 μg/ml) and penicillin G (100 units/ml). Thiamine uptake was initiated by the addition of [^{14}C]thiamine (24.3 Ci/mol, Amersham International, U.K.) and was terminated by the addition of 15 ml of ice-cold medium. The medium and cell pellets were separated by centrifugation for 5 s at $700 \times g$. The medium was aspirated and the cell pellets were washed with 10 ml of iced medium, and then centrifuged for 5 s at $700 \times g$ as described previously [4]. Blank tubes were routinely determined as follows: [^{14}C]thiamine was added to the cell suspensions at 0°C, and then immediately diluted, centrifuged and washed following the procedure described above. Cell pellets were extracted with 1 ml of 6.3% trichloroacetic acid, and the radioactivity in the extract was measured in a liquid scintillant containing Triton X-100 by liquid scintillation spectrometry. The intracellular water space was determined as the difference of ^3H_2O and [^{14}C]inulin spaces in the cell pellet [3] for each experiment. The intracellular water space was 2.63 ± 0.222 μl/10^6 cells ($n = 45$, mean $\pm$S.E.). For the experiments transport rates are presented as means $\pm$S.E. Kinetic parameters (K_t and V_{max}) and S.E. for these values were calculated as described by Wilkinson [9]. Significant differences were assessed by Student's two-tailed t-test. The structural formulae of quaternary ammonium compounds examined in this paper are presented in Fig. 1.

Table I contrasts the inhibitory effect of



	R_1	R_2
(I)	$-CH_3$	$-CH_3$
(II)	$-C_6H_5$	$-CH_3$
(III)	$-CH_2C_6H_5$	$-CH_3$
(IV)	$-CH_2C_6H_5$	$-CH_2C_6H_5$
(V)	$-(CH_2)_{15}CH_3$	$-CH_3$
(VI)	$-CH_2CH_2OOC(CH_2)_{14}CH_3$	$-CH_3$

Fig. 1. Structural formulae of quaternary ammonium compounds with and without phenyl groups.

TABLE I

EFFECT OF QUATERNARY AMMONIUM COMPOUNDS WITH AND WITHOUT PHENYL GROUPS ON THIAMINE UPTAKE

The [^{14}C]thiamine uptake was assayed as described in the text. Quaternary ammonium compounds (50 μM) were added to cell suspensions simultaneously with 10 μM [^{14}C]thiamine, and the mixtures were incubated for 30 s. The data presented are corrected for the contribution of nonsaturable uptake. The distribution ratio is the molar ratio of intracellular thiamine to thiamine in the medium. The results presented are the means $\pm$S.E. of three experiments.

Addition	[^{14}C]Thiamine uptake (pmol/10^5 cells per 30 s)	Distribution ratio	Percent
None	3.404 ± 0.068	1.324 ± 0.027	100
I	3.228 ± 0.149	1.256 ± 0.057	95
II	1.496 ± 0.121	0.582 ± 0.047	44
III	0.975 ± 0.097	0.379 ± 0.037	29
IV	0.143 ± 0.019	0.056 ± 0.007	4
V	1.221 ± 0.053	0.475 ± 0.021	36
VI	3.214 ± 0.311	1.250 ± 0.121	94

quaternary ammonium compounds containing and lacking a phenyl group. Among the compounds tested, the tetramethylammonium ion (compound I in Table I) was the least potent inhibitor of hepatocyte thiamine uptake. At the concentration 50μM the phenyltrimethylammonium ion (II), which has a phenyl group in place of the methyl group, inhibited thiamine uptake by 56%. Benzyltrimethylammonium (III) and dibenzyldimethylammonium (IV) ions inhibited uptake by 71% and 96%, respectively. The cetyltrimethylammonium ion (V) was a less effective inhibitor of thiamine uptake than the benzyltrimethylammonium ion (III). The palmitoylcholine ion (VI) was also a weak inhibitor. These results indicate that the lipophilic nature of the quaternary ammonium compounds does not account for the differences in the inhibition of thiamine uptake. The lipophilicity of their compounds was chemically determined using reversed-phase thin-layer chromatography (RP-18 F_{254}, E. Merk, Darmstadt) in 100% methanol or a solvent system of acetone and 0.2 M ammonium acetate adjusted to pH 4.8 with acetic acid, 4 : 1 (v/v). The dibenzyldimethylammonium ion, IV, a lipophilic cation, was the most potent inhibitor. The observation that ions II and III were more potent inhibitors than the nonphenyl-containing

ion, I, suggests that the presence of a phenyl group has a significant role in the inhibition of the compound on hepatocyte thiamine uptake. This suggestion is further strengthened by the observation that hemicholinium-15, hemicholinium-3 and curare, which are potent inhibitors of thiamine uptake [5], also contain phenyl groups.

To elucidate further the inhibitory effect of the dibenzyldimethylammonium ion, IV, on hepatocyte thiamine uptake, a kinetic analysis was performed as shown in Fig. 2. At the concentration 0.5 μM, ion IV was a competitive inhibitor of thiamine uptake. The ion IV increased K_t from 58.1 ± 7.23 to 106 ± 12.8 μM ($p < 0.001$), but failed to change V_{max} significantly (17.6 ± 1.10 versus 18.0 ± 1.33 pmol/10^5 cells per 30 s (n.s.)). For this inhibitor, the K_i was 0.64 μM. Since this ion at 50 μM almost completely inhibited 10 μM thiamine uptake, it is likely that the dibenzyldimethylammonium ion is a purely competitive inhibitor of thiamine uptake in isolated rat hepatocytes. These results suggest further that ion IV shares a common binding site for thiamine uptake, as previously demonstrated for choline analogs [5]. This inhibitory effect of ion IV on thiamine uptake by isolated rat hepatocytes is consistent with the inhibition reported previously in yeast cells [8]. The apparent affinity of ion IV for the thiamine carrier is 1500-fold that of ion I [5], with a K_i 1/50th that of thiamine [4]. These findings support the concept that the presence of a phenyl group enhances the inhibitory effect of quaternary ammonium compounds on thiamine uptake in isolated rat hepatocytes. It may be speculated that this enhancement is not due to nonspecific hydrophobic interaction with an amino acid residue in the thiamine carrier.

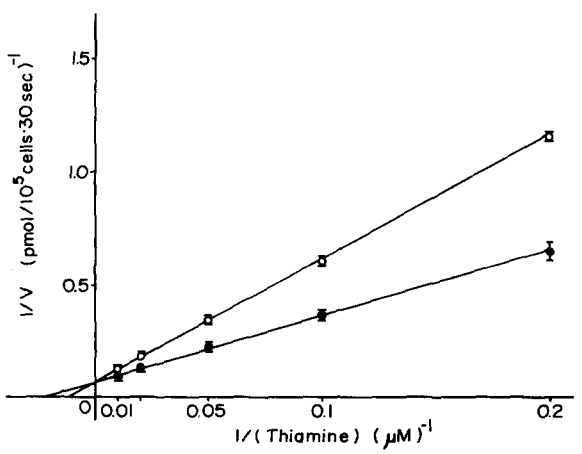


Fig. 2. Lineweaver-Burk analysis of thiamine uptake in the absence (-●-) and presence (-○-) of 0.5 μM dibenzyldimethylammonium (IV). The data presented are corrected for the contribution of nonsaturable uptake. Each value is the mean $\pm$S.E. of three experiments.

To examine further the relationship of the lipophilic nature of phenyl-containing ions to their affinity for the thiamine carrier, we compared the inhibitory effect of ion I to a series of phenyl-containing quaternary ammonium ions, $(CH_3)_3N^+(CH_2)_nC_6H_5$ ($n = 0$–6) [7] (Fig. 3). The maximal inhibitory effect occurred at $n = 5$. This finding indicates that lipophilicity is not consistently correlated with affinity for thiamine-carrier binding in isolated rat hepatocytes, and there is an optimal carbon chain length separating the phenyl group and the quaternary nitrogen atom. From the findings described above, we believe that a hydro-

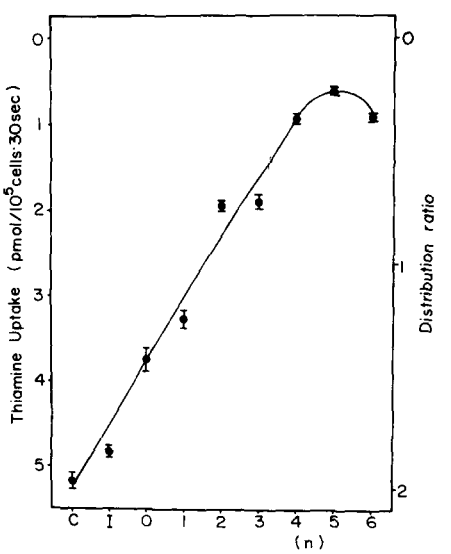


Fig. 3. Effect of tetramethylammonium (I) and carbon chain length of a series of $(CH_3)_3N^+(CH_2)_nC_6H_5$ ($n = 0$–6) compounds on thiamine uptake. C represented control. The uptake of [^{14}C]thiamine was assayed as described in the text. Ion I and the series of compounds $(CH_3)_3N^+(CH_2)_nC_6H_5$ (10 μM) were added to the cell suspensions simultaneously with 10 μM [^{14}C]thiamine and incubated for 30 s. The data were corrected for the contribution of nonsaturable uptake. The distribution ratio is the molar ratio of intracellular thiamine to thiamine in the medium. The results are the means $\pm$S.E. of three experiments.

phobic interaction of the phenyl group of quaternary ammonium compounds with the thiamine transport site enhances the affinity of these compounds for the thiamine transport site in isolated rat hepatocytes.

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