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THE STIMULATING AND INHIBITORY EFFECT OF CARBAMOYLCHOLINE ON DECAMETHONIUM UPTAKE BY SLICES OF MOUSE KIDNEY

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Abstract—The present study was performed to decide whether a common mechanism is involved in the accumulation by mouse kidney slices of the quaternary ammonium compounds decamethonium and carbamoylcholine. Slices were incubated (1 hr) in Krebs–Ringer bicarbonate medium (37°, pH 7·4) containing [^{14}C]decamethonium (2×10^{-6}M) in presence or absence of carbamoylcholine. The decamethonium uptake was significantly increased by 10^{-4}M–3×10^{-3}M carbamoylcholine, whereas 2×10^{-2}M and 3×10^{-2}M carbamoylcholine significantly inhibited the uptake. Initial decamethonium uptake (3 min incubation) was significantly stimulated when slices were preincubated (1 hr) with 3×10^{-3}M carbamoylcholine before transfer to a carbamoylcholine-free medium containing decamethonium. This suggests a relationship between the stimulating effect and the presence of carbamoylcholine in the slices. No efflux of decamethonium (incubation period 15 min) occurred from slices preincubated (1 hr) with decamethonium (2×10^{-6}M), which means that stimulation cannot be attributed to inhibition of decamethonium efflux by carbamoylcholine. The above results support the assumption that decamethonium and carbamoylcholine share a common transport mechanism. The stimulation phenomenon can be interpreted as an example of accelerative exchange diffusion, which should mean that carbamoylcholine efflux accelerates decamethonium influx.

ORGANIC bases (cations) are secreted by tubule cells in mammalian and avian kidney (see review by Peters[1]). It has also been demonstrated that organic cations accumulate in renal slices by specialized processes in many respects similar to those involved in the tubular transport of these substances in the intact kidney.[1]

Studies in our laboratory on renal transport of quaternary ammonium compounds acting on cholinergic mechanisms have demonstrated that mouse kidney slices accumulate both decamethonium and carbamoylcholine as the unchanged compounds by saturable energy dependent processes.[2,3] We have thus found it of interest to investigate whether decamethonium and carbamoylcholine share a common transport mechanism in the mouse kidney *in vitro*.

The present paper, which describes the effect of carbamoylcholine on [^{14}C]decamethonium uptake by slices of mouse kidney provides strong evidence that these two quaternary ammonium compounds share a common transport mechanism involving a mobile carrier.

MATERIALS AND METHODS

The following compounds were used: [^{14}C]methyldecamethonium dibromide (Radiochemical Centre, Amersham, England), with a specific activity of 15 mc/mM and inulin-carboxyl-^{14}C (New England Nuclear Corp., U.S.A.) with a specific activity of 1·95 mc/g. In addition unlabelled carbamoylcholine chloride (Ph.Nord. 1963) was used.

Experimental procedure

Slices of mouse kidneys were prepared and used as previously described.[2] Male albino mice of a single strain (NMRI) with a body weight of 28–32 g were decapitated and bled. Each kidney was cut into four or five slices with a razor blade. Each of the experiments recorded in Tables 1–4 was carried out as a paired comparison with kidney tissue from two animals. Eight to ten slices (total wet weight: 150 mg) from two kidneys (one kidney from each animal) were placed in a test tube, which contained 20 ml Krebs–Ringer bicarbonate solution with glucose (11 mM/l.). This procedure made it possible to incubate kidney tissue from each animal (distributed in two test tubes) simultaneously under different experimental conditions (with or without carbamoylcholine). Each animal thus served as its own control (method of paired comparison[4]).

TABLE 1. EFFECT OF CARBAMOYLCHOLINE ON THE UPTAKE (S/M RATIO AFTER 1 hr INCUBATION) OF [^{14}C]DECAMETHONIUM (2×10^{-6}M) BY SLICES OF MOUSE KIDNEY

Carbamoylcholine conc. (M)	S/M ratio		Difference (%)
	Control	Carbamoylcholine added	
10^{-5}	12·2 ± 0·3	12·8 ± 0·3	+ 5 ± 3
10^{-4}	12·2 ± 0·8	15·8 ± 1·0	+ 31 ± 8*
10^{-3}	11·8 ± 0·8	17·3 ± 0·9	+ 47 ± 4‡
3×10^{-3}	13·7 ± 0·7	17·6 ± 0·6	+ 31 ± 6†
10^{-2}	10·8 ± 0·8	10·4 ± 0·4	− 1 ± 9
2×10^{-2}	10·5 ± 0·7	8·0 ± 0·3	− 23 ± 5†
3×10^{-2}	14·6 ± 0·5	7·4 ± 0·3	− 49 ± 2‡

Slices were incubated either with or without (control) carbamoylcholine as stated in text. Results are given as the mean of values from six experiments (nine experiments with 3×10^{-3}M carbamoylcholine) ± S.E.M.

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

TABLE 2. INITIAL UPTAKE (S/M RATIO AFTER 3 min INCUBATION) OF [^{14}C]DECAMETHONIUM (2×10^{-6}M) BY MOUSE KIDNEY SLICES PRELOADED WITH CARBAMOYLCHOLINE

S/M ratio		Difference (%)
Control	Preloaded with carbamoylcholine	
1·11 ± 0·04	1·31 ± 0·06	+ 18 ± 2*

Slices were preincubated either with (preloaded) or without (control) 3×10^{-3}M carbamoylcholine for 1 hr as stated in text. Results are given as the mean of values from six experiments ± S.E.M.

* $P < 0.001$.

TABLE 3. EFFECT OF CARBAMOYLCHOLINE ON INITIAL UPTAKE (S/M RATIO AFTER 3 min INCUBATION) OF [^{14}C]DECAMETHONIUM (2×10^{-6}M) BY SLICES OF MOUSE KIDNEY

Carbamoylcholine conc. (M)	S/M ratio		Difference (%)
	Control	Carbamoylcholine added	
3×10^{-3}	1·21 ± 0·09	1·25 ± 0·09	+ 5 ± 9
3×10^{-2}	1·18 ± 0·09	0·79 ± 0·07	− 33 ± 4*

Slices were incubated either with or without (control) carbamoylcholine as stated in text. Results are given as the mean of values from six experiments ± S.E.M.
* $P < 0.001$.

TABLE 4. EFFECT OF EXTERNAL CARBAMOYLCHOLINE ON THE INITIAL UPTAKE (S/M RATIO AFTER 3 min INCUBATION) OF [^{14}C]DECAMETHONIUM (2×10^{-6}M) BY MOUSE KIDNEY SLICES PRELOADED WITH CARBAMOYLCHOLINE (3×10^{-3}M)

S/M ratio		
Control	With carbamoylcholine	Difference (%)
1·01 ± 0·06	0·76 ± 0·01	− 24 ± 5*

Experiments were performed exactly as those in Table 2 with the exception that slices, which were preloaded with carbamoylcholine, were incubated in the presence of 3×10^{-2}M carbamoylcholine. Results are given as the mean of values from six experiments ± S.E.M.
* $P < 0.01$.

Unless otherwise stated the slices were incubated for 10 min at 37° (pH 7·4) before addition of drugs. During all incubations the media were gassed with a mixture of oxygen–carbon dioxide (95:5, v/v%). A mixing of the medium was ensured by shaking the tubes (60 oscillations/min). The same amount of [^{14}C]decamethonium (0·5 μc) was added to each tube.

In the experiments recorded in Tables 1 and 3 carbamoylcholine was added to the medium just before [^{14}C]decamethonium. In the experiments recorded in Tables 2 and 4 slices were incubated either with (preloaded) or without (control) carbamoylcholine for 1 hr before being transferred to a final incubation medium, which contained [^{14}C]decamethonium. At the end of the incubation period the slices were separated from the media by filtration on PVC covered fibre glass nets placed upon cotton. Slices were transferred to another medium by submerging of nets plus slices. Otherwise, the slices were weighed (wet weight) immediately after the separation procedure.

Measurement of radioactivity

Samples of tissue and medium were prepared for radioactivity measurements in a Packard Tri-Carb liquid scintillation spectrometer (model 3375) as previously described.[2]

The results were expressed as the slice-to-medium (S/M) concentration ratio of ^{14}C, which was calculated as the counting rate per gram slice (post incubation wet weight)/ counting rate per millilitre medium.

The difference between S/M ratio in control experiment and experiment with carbamoylcholine was expressed as per cent of control value. The significance of these differences was estimated by Students *t*-test.[4]

RESULTS

Effect of carbamoylcholine on decamethonium uptake

Table 1 shows the effect of carbamoylcholine on the uptake of [^{14}C]decamethonium (2×10^{-6}M). The uptake was expressed as the slice-to-medium (S/M) concentration ratio after incubation for 1 hr.

It is seen that the decamethonium uptake is significantly increased in the presence of 10^{-4}M, 10^{-3}M and 3×10^{-3}M carbamoylcholine. The uptake was stimulated to the highest extent (50 per cent) by 10^{-3}M carbamoylcholine. The presence of either 10^{-5}M or 10^{-2}M carbamoylcholine had no significant effect on decamethonium uptake. The uptake was significantly inhibited by 2×10^{-2}M and 3×10^{-2}M carbamoylcholine. Thus, 2×10^{-2}M carbamoylcholine reduced the uptake by almost one fourth, while the uptake was halved by 3×10^{-2}M carbamoylcholine.

Initial decamethonium uptake by slices preloaded with carbamoylcholine

Table 2 shows the initial [^{14}C]decamethonium uptake by slices incubated (preloaded) for 1 hr with 3×10^{-3}M carbamoylcholine before transfer to a carbamoylcholine free medium with [^{14}C]decamethonium (2×10^{-6}M). The uptake is expressed as slice-to-medium (S/M) concentration ratio after incubation for 3 min. It is seen that preloading with carbamoylcholine significantly increases initial decamethonium uptake.

The size of the extracellular space of the slices was estimated by determining the slice-to-medium concentration ratio (1 hr of incubation) of [^{14}C]inulin (10^{-6}M), which was $0{\cdot}39 \pm 0{\cdot}02$ (mean value $\pm$ S.E.M. from six experiments). It should be mentioned that the simple distribution of decamethonium in the extracellular space of the slices must constitute a great part of the total decamethonium uptake in short incubation experiments. This might explain that the percentage differences (inhibition or stimulation with carbamoylcholine) are somewhat lower in initial uptake experiments (Tables 2–4) than in 1 hr uptake experiments (Table 1).

Effect of carbamoylcholine on initial decamethonium uptake

Table 3 shows the effect of carbamoylcholine (3×10^{-3}M and 3×10^{-2}M) on initial decamethonium uptake. The uptake of decamethonium (2×10^{-6}M) was expressed as the S/M ratio after incubation for 3 min. It is seen that initial decamethonium uptake is not significantly affected by 3×10^{-3}M carbamoylcholine, whereas a tenfold higher concentration of carbamoylcholine reduces the uptake significantly by one third.

Effect of external carbamoylcholine on initial decamethonium uptake by slices preloaded with carbamoylcholine

Table 4 shows the effect of external carbamoylcholine on initial [^{14}C]decamethonium uptake by slices, which were preloaded (1 hr) with 3×10^{-3}M carbamoyl-

choline and then transferred to a medium containing [^{14}C]decamethonium (2×10^{-6}M) and carbamoylcholine (3×10^{-2}M). The uptake is expressed as S/M ratio after incubation for 3 min.

It is seen that the stimulation of initial decamethonium uptake, which occurs in slices preloaded with carbamoylcholine (Table 2), completely disappears when 3×10^{-2}M carbamoylcholine is present in the external medium. On the contrary, the initial decamethonium uptake is significantly reduced in this type of experiment as compared to control experiment.

Efflux of decamethonium

Slices were incubated (1 hr) with 2×10^{-6}M [^{14}C]decamethonium and then transferred to a decamethonium free medium (20 ml). Following incubation for 15 min in this medium the decamethonium S/M ratio was found to be 13·0 $\pm$ 0·4 (the mean $\pm$ S.E.M. of values from six experiments). This is the same as the S/M ratio found after incubation for 1 hr with 2×10^{-6}M decamethonium (Table 1).

DISCUSSION

The present experiments show that the 1 hr uptake of decamethonium (2×10^{-6}M) by slices of mouse kidney is increased by 10^{-4}M, 10^{-3}M and 3×10^{-3}M carbamoylcholine, whereas 2×10^{-2}M and 3×10^{-2}M carbamoylcholine reduces the uptake. The increased initial decamethonium uptake by slices preloaded with 3×10^{-3}M carbamoylcholine (Table 2) suggests that the stimulation is related to the presence of carbamoylcholine in the slices. That 3×10^{-3}M carbamoylcholine added to medium just before decamethonium did not increase initial decamethonium uptake (Table 3) is consistent with this suggestion. The data (Table 3) also show that 3×10^{-2}M carbamoylcholine added to medium just before decamethonium inhibited initial decamethonium uptake, which suggests that this effect of carbamoylcholine is related to its presence in external medium. The increase in initial decamethonium uptake cannot be attributed to a competitive inhibition of decamethonium efflux by internal carbamoylcholine, as no efflux was shown to occur from slices preloaded with decamethonium. The presence of carbamoylcholine at a high concentration (3×10^{-2}M) in external medium inhibits initial decamethonium uptake by slices preloaded with carbamoylcholine (Table 4), which means that external carbamoylcholine suppresses the ability of internal carbamoylcholine to stimulate decamethonium uptake.

Several papers have appeared in recent years reporting accelerated flux phenomena in association with transport of hexoses and amino acids. For instance, Levine, Oxender and Stein,[5] who studied the efflux of [^{3}H]glucose from human erythrocytes into a medium containing varying concentrations of unlabelled glucose, found an increase in the rate of efflux of [^{3}H]glucose as the external glucose level was increased. Furthermore, the rate of [^{3}H]glucose exit into a galactose medium was higher than that into a galactose-free medium. These results were interpreted as examples of substrate-facilitated carrier transport or accelerative exchange diffusion, which means that the rate of movement across the membrane of the substrate–carrier complex is supposed to be greater than that of the free carrier. The flux of a substrate in one direction (influx of unlabelled glucose or galactose) should thus accelerate the flux of the same or another substrate in the opposite direction (efflux of [^{3}H]glucose) by

increasing the rate of return of the carrier (as a substrate–carrier complex) from external to internal side or vice versa. Heinz and Walsh,[6] who studied the uptake of [^{14}C]glycine by Ehrlich mouse ascites carcinoma cells, found that preloading the cells with unlabelled glycine accelerated influx of [^{14}C]glycine. A similar effect on [^{14}C]glycine influx was obtained by preloading the cells with *N*-methylglycine, whereas this amino acid if present in external medium depressed influx of [^{14}C]glycine.

The stimulating effect of carbamoylcholine on decamethonium uptake is most likely to be interpreted as an example of accelerative exchange diffusion, which should mean that carbamoylcholine efflux accelerates decamethonium influx. This interpretation is consistent with the conclusion from the experiments on mutual inhibition that the quaternary ammonium compounds decamethonium and carbamoylcholine share a common carrier mechanism in the mouse kidney. The weak inhibition of decamethonium uptake seen with carbamoylcholine suggests that the latter agent has a low affinity to the carrier as compared to that of decamethonium.

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