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Augmentation of peritoneal dialysis clearance with procaine

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Augmentation of peritoneal dialysis clearance with procaine. Local anesthetic procaine in a 0.25% concentration was used as the adjunct to the dialysis fluid during peritoneal dialysis in rabbits. Procaine increased peritoneal clearance of urea (2.213 ± 0.187 ml/min to 3.544 ± 0.41 ml/min) and inulin (0.528 ± 0.079 ml/min to 0.852 ± 0.213 ml/min). Effect of the drug persisted, despite its lack, during the following cycles. Procaine influenced transmesothelial transfer of urea and inulin in vitro. The effect of the drug was biphasic. During the first 20 min a decrease of both solutes fluxes was observed. Then the transport rate of urea and inulin started to grow. The effect of procaine was independent of the membrane's side to which the drug was added. At least two sites of procaine action (vascular system and mesothelium) during peritoneal dialysis are proposed.

Augmentation de la clearance de dialyse péritonéale avec la procaïne. La procaïne, un anesthésique local, à la concentration de 0,25% a été employée comme adjuvant au liquide de dialyse pendant une dialyse péritonéale chez des lapins. La procaïne a augmenté la clearance péritonéale de l'urée (de 2,213 ± 0,187 ml/min à 3,544 + 0,41 ml/min) et de l'inuline (de 0,528 ± 0,079 ml/min à 0,852 ± 0,213 ml/min). L'effet du médicament a persisté, même en son absence, pendant les cycles suivants. La procaïne a influencé le transfert transmésothélial de l'urée et de l'inuline in vitro. L'effet du médicament était biphasique. Pendant les 20 premières minutes une diminution du flux des deux solutes était observé. La vitesse de transport de l'urée et de l'inuline commençait ensuite à croître. L'effet de la procaïne était indépendant du côté de la membrane où le médicament était ajouté. On propose au moins deux sites d'action de la procaïne (système vasculaire et mésothélium) pendant la dialyse péritonéale.

Peritoneal dialysis is a recognized form of treatment in patients with renal failure [1, 2]. However, its use is not widespread due to its believed limited efficiency in comparison with hemodialysis [3]. A number of approaches have been made in an attempt to increase peritoneal dialysis efficiency [4–7]. Recently, data describing the changes of the mesothelial permeability to solutes under the influence of docusate sodium or protamine have been presented [8, 9].

Local anesthetics can change both the structure and function of cells and epithelia [10–12]. Precise mechanism of their action at the molecular level is not known, however, interaction with lipids of the cellular membrane or dissociation of the cytoskeletal elements were suggested [13, 14]. Besides, they can cause vasodilation of the blood vessels [15, 16]. The purpose of our study was to find the effect of the local anesthetic procaine, used intraperitoneally, on the solute clearances during peritoneal dialysis. To distinguish between the vascular and mesothelial site of the drug action, we performed experiments on rabbits in vivo and on the isolated fragments of the rabbit's mesentery in vitro.

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Methods

Experiments in vivo. Peritoneal dialysis was performed in seven healthy New Zealand white rabbits weighing an average of 2.2 kg (1.8 to 2.5 kg). The animals were anesthetized with ketamine (Ketanest, Parke-Davis, Morris Plains, New Jersey), 15 mg/kg of body wt i.m., and Diazepam (Relanium-Polfa), 1 mg/kg of body wt i.m., in preparation for the cannulization of blood vessels and placement of the dialysis catheter. During the performance of the peritoneal dialysis, rabbits were sedated with Diazepam, 0.01 mg/kg body wt/min i.v. The dialysis catheter was placed in the right lower quadrant of the abdomen. To avoid leaks peritoneum and abdominal muscles were sutured in two layers. Any leak of dialysis fluid or persistent bleeding excluded the animal from the experimental protocol. Femoral vein and artery were cannulated. To maintain a constant blood level of urea and inulin, a primary dose of urea, 10 mg/100 g body wt, and inulin, 13.75 mg/100 g body wt, was followed by a continuous infusion of saline solution containing urea (20 mg%/100 g body wt) and inulin (27 mg%/100 g body wt) with the rate of 0.48 ml/min.

Peritoneal dialysis was performed with Hanks solution made hypertonic (370 mOsm) with mannitol. Heparin was added into the fluid (50 U/dl) to prevent the catheter obstruction by fibrin. The dialysate exchange volume was 75 ml/kg body wt. Dialysis fluid was warmed to 37°C and instilled into the peritoneal cavity within 1 min with a syringe. Instantly after the instillation, samples of dialysate and arterial blood were taken for urea and inulin level measurements. Dwell-time was 20 min and after that time blood and dialysate samples were taken again. Then dialysate was allowed to drain by gravity; drainage was assisted by applying light manual massage to the abdomen. Urea concentration in samples was estimated by the diacetyl monoxime method [17]. Absorbance of the colored reaction product was measured on a spectrophotometer (Spectromom 203-MOM, Hungary). Inulin concentration was measured by the anthrone method [18].

In every rabbit dialysis consisted of seven cycles. Three preliminary cycles were designated control cycles and the following as experimental. Procaine (Procaine-HCl-Koch Light Laboratory) was added to the dialysis fluid in cycles 4 and 7 to achieve its final

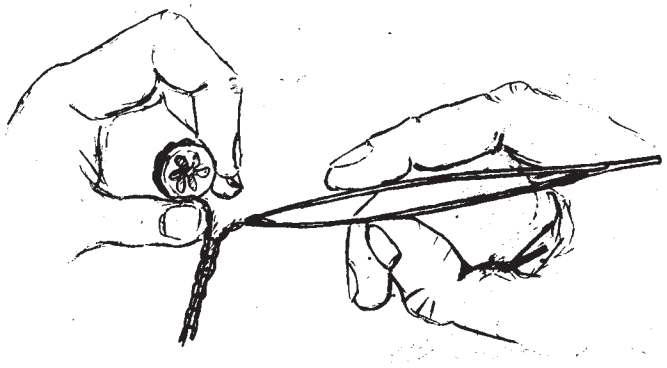

Fig. 1. *The preparation of the mesentery to obtain a single layer of the mesothelium.*

concentration of 0.25%. Solute clearances were calculated for each dialysis exchange according to the equation:

$$\text{Clearance}_{\text{ml/min}} = \frac{V_d \times C_{d2} - C_{d1}}{\left[\frac{C_{s1} + C_{s2}}{2} - C_{d1}\right] \times t} \qquad (1)$$

where: C_{d1} and C_{d2} represent the concentration of the solute in the dialysate at the beginning and at the end of the exchange, respectively; V_d, volume of dialysate recovered; C_{s1} and C_{s2}, concentration of the solute in serum at the beginning and at the end of the exchange, respectively; t, dwell-time.

A separate series of experiments was performed during which no drug was added to the dialysis fluid. The purpose of this study was to evaluate the changes of the peritoneal permeability in time.

Experiments in vitro. Studies were performed on the mesentery of the ileum from the New Zealand white rabbits. The animals were killed by a blow on the head. Then the abdomen was opened and the ileal mesentery was isolated. A procedure was performed to obtain the membrane possessing only one layer of the mesothelium. The mesentery was dissevered by a gentle mechanical preparation in situ (Fig. 1). The obtained membrane, containing a single layer of the mesothelium, was mounted between two semichambers with active area 1.3 cm^2. Chambers were filled with Hanks solution which was mixed, oxygenated, and buffered with a gas mixture (98% O_2 and 2% CO_2). Inulin and urea were added to the vascular side of the membrane to obtain the concentration of 50 and 100 mg%, respectively. Every 10 min samples were taken from both sides of the membrane. The urea concentration was measured in the same manner as in in vivo experiments. The transfer of inulin was evaluated with the use of the isotope tracer (^{3}H-inulin, Amersham, Arlington Heights, Illinois) added simultaneously to the vascular side of the membrane with the cold substance. Radioactivity of the samples was measured with a beta counter (LS 100C Beckman Instruments Inc., Fullerton, California). Values of urea and inulin transfer were corrected for the steadily declining concentration gradient between the vascular and the mesothelial side of the membrane. To assess the viability of the mesothelial cells, a series of experiments was performed in which transepithelial transport of urea and inulin was evaluated for a duration of 100 min.

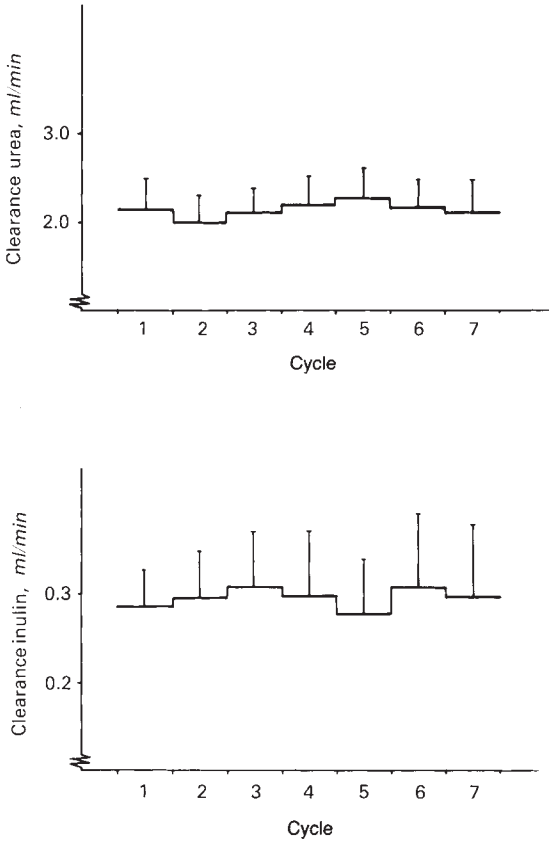

Fig. 2. *Mean values ± SE of peritoneal clearances of urea and inulin during dialysis performed without procaine.*

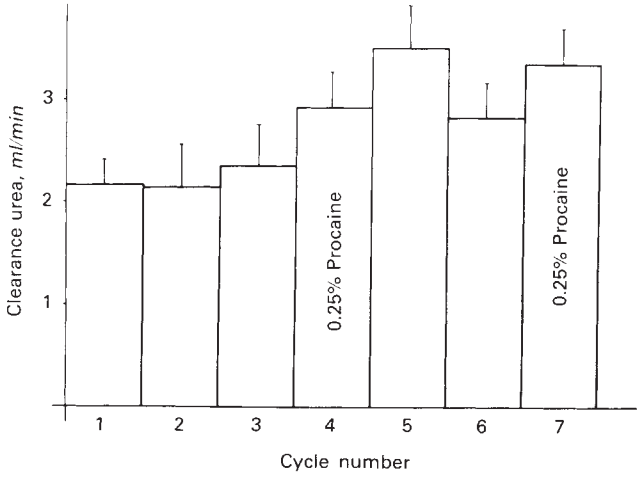

Fig. 3. *Mean values ± SE of peritoneal clearance of urea during dialysis performed with procaine.*

During experiments with procaine every membrane studied served as its own control. Control fluxes were measured for 30 min. After that time procaine was added either to the mesothelial or the vascular side of the membrane in an amount sufficient to obtain the final concentration in the 0.25% medium.

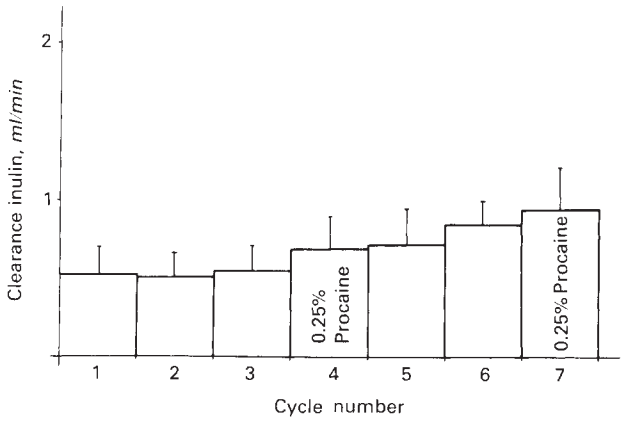

Fig. 4. *Mean values ± SE of peritoneal clearance of inulin during dialysis performed with procaine.*

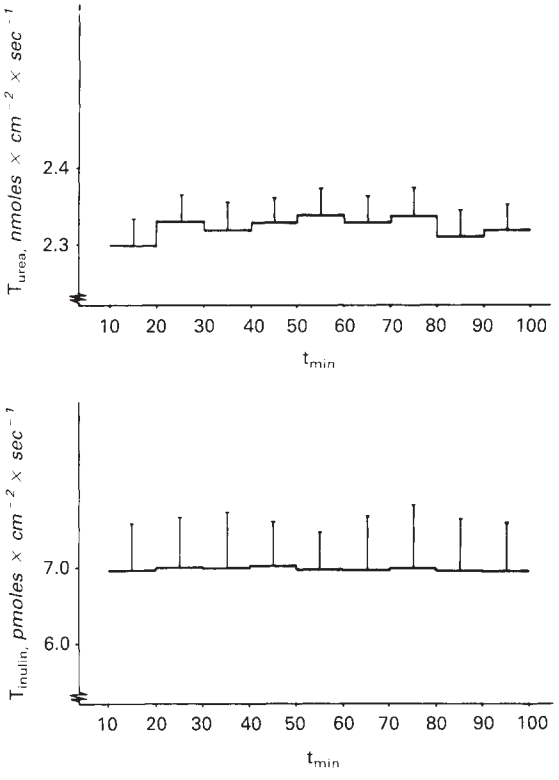

Fig. 5. *Mean values ± SE of the transmesothelial transport of urea and inulin in vitro in sham experiments.*

Then the effect of the drug on the transmesothelial transport of the studied solutes was observed for 50 min.

Results were expressed as mean ± SE. Student's *t* test for paired data was used. A *P* value of less than 0.05 was considered significant.

Results

Experiments in vivo. During control experiments performed without the anesthetic, the values of the peritoneal clearances of urea and inulin did not change significantly in time (Fig. 2).

In procaine experiments mean values of urea and inulin peritoneal clearances during three preliminary dialysate exchanges were 2.213 ± 0.187 and 0.529 ± 0.079 ml/min, respectively. Procaine added to the dialysis fluid increased the clearance of urea by 25% on the average ($P < 0.01$). The effect persisted during the next cycle, which was performed without an anesthetic, because the urea clearance was higher by 53% in reference to the control ($P < 0.01$). After that time the effect of procaine on the urea peritoneal removal diminished; in cycle 6, in which dialysis fluid was still without procaine, urea clearance was higher than in control cycles by only 22% ($P < 0.05$). Application of the drug during the next cycle again caused an increase of the urea peritoneal clearance (Fig. 3).

Procaine added intraperitoneally also augmented the removal of inulin via peritoneum. The increase was instant, however, the effect was slower and prolonged (Fig. 4). The maximal effect was observed in cycle 6 in which inulin clearance was higher by 54% in reference to the control period ($P < 0.05$). Average values ± SE of urea and inulin peritoneal clearances in all cycles are listed in Table 1.

Experiments in vitro. We conducted a separate series of control experiments without any drug and did not observe significant changes of the transmesothelial transport of urea and inulin (Fig. 5).

Procaine applied either to the mesothelial or to the vascular side of the membrane affected permeability of the mesothelium similarly. Initially, a decrease of both fluxes was observed. However, after 20 min the transport rate of urea and inulin began to rise (Fig. 6). Mean values ± SE of urea and inulin transfer in every period studied are presented in Table 2. Transmesothelial transport of urea was in each case more inhibited during the first phase of the drug's action, than during that of inulin. However, in the second phase of the drug's action, the maximal increase of the transepithelial transfer of inulin was higher than the respective measure for urea.

Discussion

Based upon the results obtained we conclude that local anesthetic procaine in a concentration of 250 mg/dl of the dialysis fluid does increase the peritoneal clearance of urea and inulin. The effect is instant and retentive. It results from the in vivo as well as from the in vitro studies, which showed that peritoneal clearances and flux rates across the membrane rose constantly after the drug was added to the bathing fluid.

There may be at least two sites of procaine action during peritoneal dialysis. Procaine presumably increases blood flow into the peritoneal microcirculation either by the blockade of the autonomic nerve fibers or due to its direct vasodilating action [15, 16]. However, another mechanism of action, that is, the increase of the mesothelial permeability, should also be considered.

During the first exchange performed with the anesthetic, the enhancement of urea and inulin peritoneal clearances was roughly the same (25 and 23%, respectively). In the subsequent fluid exchange, performed without procaine, urea clearance was increased by 54% in reference to control whereas inulin clearance was increased by only 25%. This discrepancy may be interpreted as an increase of the total membrane area available for diffusion which may be due to the vasodilation. During the next exchange, which was performed also without the anes-

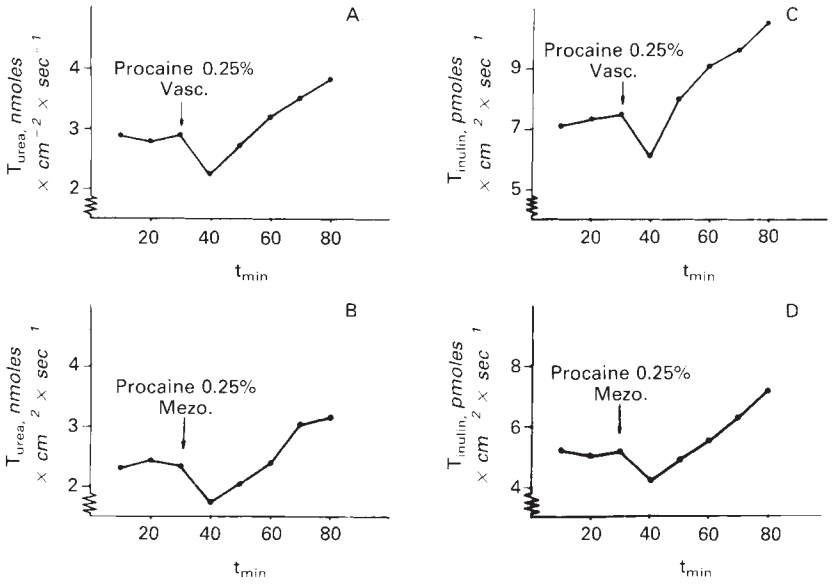

Fig. 6. *Transmesothelial transfer of urea and inulin in vitro in experiments with procaine.*

Table 1. Average peritoneal urea and inulin clearances ± SE during dialysis performed with procaine

Clearance of substance studied	Cycle						
	1	2	3	4 Procaine	5	6	7 Procaine
$Clearance_{urea}$, *ml/min*	2.168 ±0.257	2.150 ±0.409	2.32 ±0.392	2.903 ±0.324 $P < 0.01$ +25%	3.544 ±0.410 $P < 0.01$ +53%	2.835 ±0.338 $P < 0.05$ +22%	3.343 ±0.335 $P < 0.01$ +44%
$Clearance_{inulin}$, *ml/min*	0.519 ±0.162	0.512 ±0.142	0.553 ±0.149	0.682 ±0.170 $P < 0.02$ +23%	0.694 ±0.140 $P < 0.05$ +25%	0.852 ±0.213 $P < 0.05$ +54%	0.943 ±0.253 $P < 0.05$ +71%

thetic, the relations were inverse; peritoneal clearance of inulin was more augmented than the respective measure for urea. Therefore, we interpreted that as an increase of the mean pore radius in the membrane. We postulate that procaine given intraperitoneally acts through two main mechanisms: vasodilation with the subsequent increase of the membrane's permeability due to the enlarged mean pore radius in the mesothelium. A similar magnitude of enhancement of the peritoneal clearances of urea and inulin during the first fluid exchange with procaine, despite the vasodilation, may be explained by a simultaneous decrease of the membrane's permeability to both solutes. During experiments in vitro it was more marked for urea and a similar effect may be expected in vivo. Therefore, an increase of the urea removal via the peritoneum due to vasodilation may be reduced by a concomitant decrease of the mesothelial permeability to this solute.

It is difficult to precisely define the nature of changes induced by procaine in the function of the mesothelium. It is postulated that local anesthetics interact with lipids of the cell membrane and with the cytoskeleton as well [13, 14]. Nicolson, Smith, and Poste [10] found that local anesthetics added to the culture of the endothelioid cells BALB/3T3 caused alterations in cell shape and cytoskeleton organization within 15 to 30 min. Local anesthetics disrupt microtubules and microfilaments; their action can be duplicated by treatment of the cells with colchicine or vinblastine together with cytochalasin B [14]. There is some evidence that the tight-junction permeability depends on the cytoskeletal function [19]. Inulin is supposed to cross the mesothelium through the paracellular pathway. During in vitro experiments we observed a greater, procaine-induced, increase of the inulin transepithelial transfer in comparison with urea. Therefore, we speculate that this effect was due to the increase of the paracellular pathway permeability.

We suppose that procaine also affects the permeability of the transcellular pathway, which could explain the greater decrease of the urea transfer in comparison with inulin during a preliminary 20-min period in in vitro experiments. Urea is believed to cross the mesothelium also through the transcellular pathway and perhaps a procaine-induced decrease of its permeability could be responsible for the observed discrepancies. This effect may be transient or it may be overshadowed by later changes in the paracellular pathway.

We cannot say ultimately on which side of the mesothelium procaine acts. During in vitro experiments, changes of the

Table 2. Mean values ± SE of the transmesothelial transfer rate of urea and inulin during in vitro experiments with procaine

Membrane's side of procaine action	Studied substance	Time, *min* 10	20	30	40	50	60	70	80
	$N = 9$								
Vascular	T_{urea}	2.911	2.825	2.934	2.268	2.759	3.189	3.532	3.850
	nmoles × cm^{-2} × s^{-1}	±0.315	±0.320	±0.279	±0.323	±0.327	±0.239	±0.358	±0.480
					$P < 0.01$	NS	NS	NS	$P < 0.05$
					−23%	−6%	+9%	+20%	+32%
	$N = 8$								
Mesothelial	T_{urea}	2.313	2.439	2.372	1.754	2.067	2.422	3.048	3.168
	nmoles × cm^{-2} × s^{-1}	±0.481	±0.241	±0.278	±0.237	±0.242	±0.238	±0.274	±0.359
					$P < 0.01$	NS	NS	$P < 0.01$	$P < 0.01$
					−26%	−13%	+2%	+28%	+34%
	$N = 9$								
Vascular	T_{inulin}	7.191	7.379	7.533	6.182	8.028	9.209	9.703	10.680
	pmoles × cm^{-2} × s^{-1}	±0.763	±1.129	±1.068	±0.932	±1.155	±1.238	±1.231	±1.368
					$P < 0.02$	NS	NS	$P < 0.05$	$P < 0.01$
					−18%	−7%	22%	+29%	+42%
	$N = 8$								
Mesothelial	T_{inulin}	5.275	5.023	5.210	4.245	4.972	5.681	6.360	7.202
	pmoles × cm^{-2} × s^{-1}	±0.787	±0.601	±0.771	±0.735	±0.779	±0.780	±0.847	±1.018
					$P < 0.05$	NS	NS	NS	$P < 0.05$
					−19%	−5%	+9%	+22%	+38%

mesothelial permeability evoked by the anesthetic were instantaneous, independently on the side of the membrane on which the drug was applied. Therefore, we speculate further that the interaction of the drug with the basement or apical cell's membrane may cause subsequent, similar changes in the function of the cytoskeletal system.

It is difficult to foresee, whether or not a local anesthetic may become useful during peritoneal dialysis in humans. But it seems that their action may be advantageous as the adjunct to the dialysis fluid, inducing a slight anesthesia of the peritoneal cavity, and providing patients with a higher level of comfort.

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