4. W. Bowen, S. Gentleman, M. Herkenham, et al., Proc. Natl. Acad. Sci. USA, 78, 4818 (1981).
5. J. Brick and G. P. Horowitz, Pharmacol. Biochem. Behav., 16, 473 (1982).
6. H. A. Feldman, Anal. Biochem., 48, 317 (1972).
7. M. V. Fischel and R. Medzihradsky, Molec. Pharmacol., 20, 269 (1981).
8. H. J. Fridman, M. B. Bass, and D. Lester, Pharmacol. Biochem. Behav., 13, 773 (1981).
9. D. B. Goldstein and J. H. Chin, Fed. Proc., 40, 2073 (1981).
10. J. M. Littleton, Adv. Biol. Psychiat., 3, 75 (1979).

PHARMACOKINETICS OF SERUM PHOSPHOCREATINE IN MAN, DOG, AND RABBIT

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Investigation of the metabolism of the ischemic heart and of ultrastructural injuries to its cells at the stage of transition of these injuries into irreversible have clearly demonstrated that the time of this transition coincides with a reduction in the concentration of high-energy phosphates below a critical level [3, 5, 6]. Phosphocreatine, which participates in the mechanism supplying energy for contraction as an intracellular carrier of energy, also has been shown to have a significant protective action on the ischemic myocardium [4, 7]. Before analogous investigations of the protective action of phosphocreatine can be conducted *in vivo*, however, further information is required on the pharmacokinetics of phosphocreatine in the blood, so that the possible effective doses of this substance can be accurately determined.

In the present investigation the pharmacokinetics of phosphocreatine in human, canine, and rabbit blood plasma was studied.

EXPERIMENTAL METHOD

Experiments were carried out on healthy rabbits and dogs anesthetized with pentobarbital (25 mg/kg body weight, intravenously). Phosphocreatine (disodium salt) in physiological saline was injected intravenously in the form of a bolus or by infusion. At the times indicated in Figs. 1 and 2, 6 ml of blood was taken from the carotid artery and kept on ice until the end of the experiment, when plasma was obtained from it by standard methods. To determine the concentrations of creatine and phosphocreatine in the plasma, it was extracted in 6% $HClO_4$ (1 ml plasma and 0.5 ml of acid) and in 35% methanol. The plasma was then centrifuged for 10 min at 8000 rpm in a U2-21 centrifuge (Beckman, USA), with UA-20 rotor. The supernatant was poured off and neutralized with 5% K_2CO_3 (to pH 7.0) and recentrifuged under the same conditions. The supernatant was again poured off and concentrations of creatine and phosphocreatine in it were determined colorimetrically [2]. Phosphocreatine was converted into free creatine by hydrolysis in 0.1 M HCl for 10 min, after which total creatine was determined colorimetrically. Free creatine was determined in parallel tests without hydrolysis, and the phosphocreatine concentration was calculated as the difference between total and free creatine.

The pharmacokinetics of phosphocreatine in man was determined by taking 6-ml blood samples from the subclavian vein by means of a catheter at times after a single intravenous injection of Neoton (Schiapparelli Farmaceutici, Turin, Italy) or after infusion of this substance into the cubital vein.

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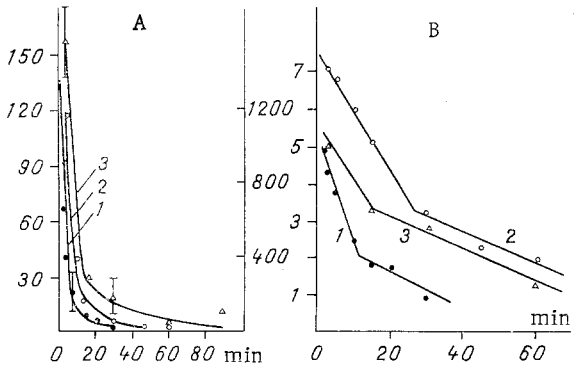

Fig. 1. Phosphocreatine clearance from blood serum after a single intravenous injection into rabbits (3) in a dose of 10 mg/kg, n = 3, into dogs (2) in a dose of 100 mg/kg, n = 3, and into human subjects (1) in a dose of 14 mg/kg, n = 6. Averaged results for 3 to 6 experiments (n) are given. Standard deviations shown for individual points, and amounted to 20% of the mean value. A. Ordinate) phosphocreatine concentration (in μM), on left) for man and rabbit, on right) for dog. Abscissa, time (in min). B. Analysis of data shown in Fig. 1A between coordinates: ordinate ln [PC]; abscissa, time (min).

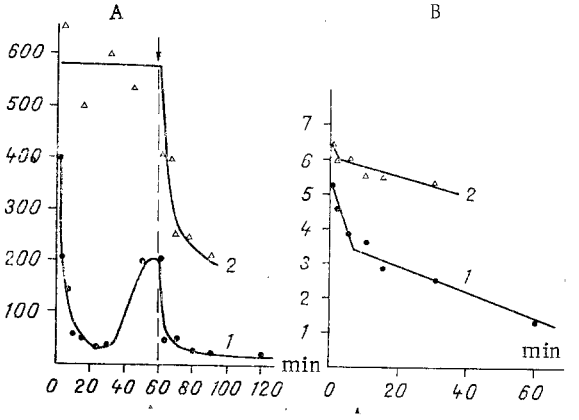

Fig. 2. Changes in phosphocreatine concentration in blood serum after intravenous infusion of a bolus of 20 mg/kg into rabbits (2), infusion of 200 mg/kg for 1 h (n = 5); into man (1), infusion of a bolus of 28 mg/kg, infusion of 56 mg/kg for 1 h (n = 7). A. Ordinate, phosphocreatine concentration (in μM); abscissa, time (min). Arrow indicates end of infusion. B. Linearization of dependence of blood phosphocreatine concentration on time. Ordinate, ln [PC]; abscissa, time (min).

EXPERIMENTAL RESULTS

After a single intravenous injection of phosphocreatine, its concentration in the blood plasma fell very rapidly to 10-20 μM in the course of 10-20 min both in the experimental animals and in man (Fig. 1). This was followed by a second phase of excretion at a slower rate. The graph of plasma phosphocreatine concentration as a function of time was linearized between coordinates in ln [PC]—time, when the relationship is precisely expressed by two straight lines with different slopes, evidence that phosphocreatine excretion is biexponential in character. By analyzing the data in this way, two velocity constants of phosphocreatine excretion (k^1_C and k^2_C), which are incorporated into a first-order equation for the clearance of this substance:

$$[PC] = [PC]_0 \left(e^{-k_B^1 t} + e^{-k_B^2 t}\right),$$

where [PC] denotes the phosphocreatine concentration at time t and $[PC]_0$ the initial phosphocreatine concentration in the plasma after administration of a single dose. The mean values

TABLE 1. Constants of Phosphocreatine Clearance from Blood Serum

Test object	Clearance constants, min^{-1}		Half-clearance time τ, min	
	k_c	k_c	τ_1	τ_2
	Single injection			
Man	0,18±0,02	0,25±0,01	5,7±0,8	27±13
Dog	0,19±0,02	0,048±0,03	4,5±1,2	28±14
Rabbit	0,16±0,03	0,025±0,003	6,8±1,1	41±8,3
	Bolus + infusion			
Man	0,22±0,05	0,015±0,005	4,60±1,1	50±21
Rabbit	0,17±0,04	0,02±0,006	5,8±1,3	76±32

Legend. Mean values and standard deviations for 3-7 experiments given.

of the constants determined in this way are given in Table 1, which also shows values of the half-clearance time ($\tau = 1/k_c$), calculated from values of the constants k_c, the result being 4-5 min for the first phase and 20-33 min for the second phase. Thus half of the phosphocreatine injected into the blood stream is eliminated in 4-5 min because of the rapid first phase, but later the PC concentration is maintained at a low level for a long time. Maintenance of a significant phosphocreatine concentration in the blood evidently requires continuous administration by infusion at a rate determined by equation (1).

Figure 2 shows that the phosphocreatine concentration can in fact be maintained at a sufficiently high and constant level in this way. In particular, if phosphocreatine is injected into man at the rate of 60 mg/h/kg body weight, the serum phosphocreatine concentration is maintained at 0.2 mM. After the end of infusion the phosphocreatine concentration falls rapidly; values of the clearance constants under these circumstances are indistinguishable from their values for a single injection (Fig. 2B; Table 1). It is not yet clear, however, what mechanism is responsible for the rapid clearance of phosphocreatine from the blood serum: storage in depots in certain tissues or elimination from the body. The results of the present investigation are evidence that in order to maintain a significant phosphocreatine concentration in the blood to achieve a pharmacologic effect, the method of continuous infusion of this substance must be used. Since experiments on rabbits showed that injection of phosphocreatine by intravenous infusion causes a substantial decrease in size of the necrotic zone in myocardial infarction [1], the data obtained in the present investigation suggest that this method of administration of phosphocreatine is optimal as regards the study of the possibility of treatment of acute myocardial infarction in man.

LITERATURE CITED

1. E. I. Chazov, N. I. Afonskaya, M. Ya. Ruda, et al., Kardiologiya (1985), in Press.
2. P. Eggliton, S. R. Elsden, and N. Gough, Biochem. J., 37, 526 (1943).
3. J. T. Flaherty, M. L. Weisfeldt, B. H. Bulkey, et al., Circulation, 65, 561 (1982).
4. D. J. Hearse, D. A. Stewart, and M. V. Braimbridge, Circulation, 54, 193 (1976).
5. K. A. Reimer, R. B. Jennings, and M. L. Hill, Circulat. Res., 49, 901 (1981).
6. K. A. Reimer, R. B. Jennings, and A. H. Tatus, Am. J. Cardiol., 52, 72A (1983).
7. L. A. Robinson, M. V. Braimbridge, and D. J. Hearse, Lancet, 1, 995 (1983).