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## Reduction of platelet adhesiveness in rabbits injected intramuscularly with acid buffers

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In the course of investigating possible effects of creatinine on platelet adhesiveness in rabbits we found that the adhesiveness of platelets in blood for glass beads was reduced by creatinine HCl injected intramuscularly but not infused intravenously, albeit the increases in plasma creatinine levels obtained with the two forms of administration were similar (Fig. 1). Subcutaneous and intraperitoneal administration failed to reduce adhesiveness.

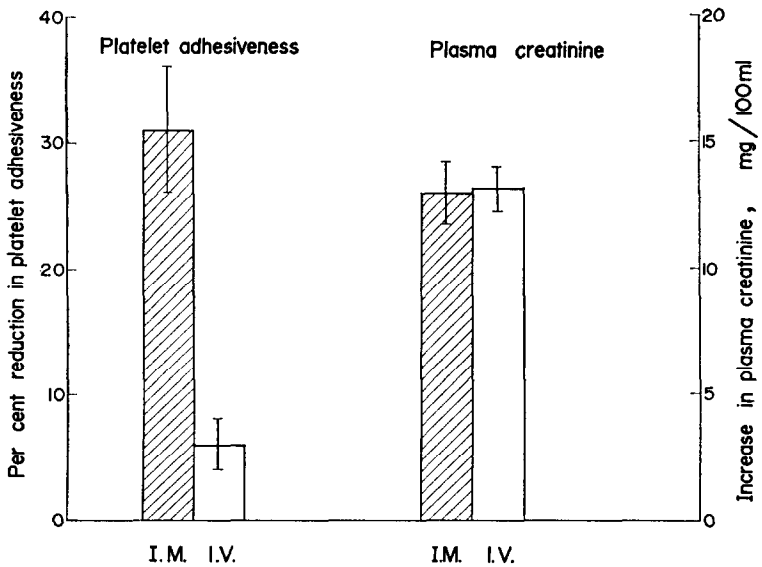


Fig. 1. Effects in the same rabbit of an intramuscular injection (100 mg/kg) or an intravenous infusion (35 mg/ml; 0·29 ml/min) of creatinine HCl on platelet adhesiveness and plasma creatinine concentrations. Mean values for six rabbits ± S.E. Blood samples were taken 15 min after the injection or start of the infusion.

Recalling that parenteral administration of ADP or ATP lowers platelet adhesiveness in rabbits[1] we thought that intramuscular administration of creatinine HCl might be releasing muscle nucleotide into the blood stream. Attempts to demonstrate ATP by the firefly assay[2] in plasma prepared from arterial blood taken from an ear of a rabbit receiving an intravenous infusion of ATP were unsuccessful. We then injected creatinine HCl intramuscularly into rabbits and determined the ATP and ADP levels in the injected muscle 10 min after administration. The average values obtained for ATP (mg/100 g wet tissue) on muscles from untreated rabbits or rabbits injected with physiological saline (1 ml/kg) or creatinine HCl (100 mg/ml/kg) were 334 ± 18 (S.E., six rabbits), 302 ± 10 (six rabbits) and 255 ± 15 (12 rabbits) respectively. The corresponding values for ADP were 32 ± 8, 27 ± 3 and 28 ± 3.

These differences in muscle ATP levels encouraged us to make further attempts to demonstrate ATP in plasma, and we succeeded in this using anaesthetised rabbits and collecting blood samples

into $K_2$EDTA via a short cannula inserted into an external iliac vein draining the tibialis muscle injected with ATP or creatinine HCl. The results indicated that an injection of creatinine HCl (100 mg/kg) resulted in an increase in plasma ATP plus ADP concentration of 3·5 ± 1·5 μM: the increases after injections of ATP in doses of 1 and 2·5 mg/kg were 1·3 ± 0·4 and 11·6 ± 2·7 respectively.

Further experiments revealed that creatinine base injected intramuscularly neither reduced platelet adhesiveness nor liberated ATP into the blood stream, hence we determined the effects of injections of a creatinine base solution adjusted to various pH values with HCl. Adhesiveness values were determined before and at 2-min intervals for 18 min after the injection. The percentage reductions in adhesiveness at the stated times were summed to give scores representing the areas under the time response curves (Fig. 2). Clearly adhesiveness was reduced by creatinine in the pH range 2–4·5 but activity was negligible at pH 5. Saline at pH 3 reduced adhesiveness in some but not all rabbits (av. score = 82 ± 34, seven rabbits) but the effects were more transient than after creatinine (263 ± 92) at this pH. Creatinine solution is strongly buffered, so we tried injecting another buffer, i.e. glycine (200 mg/ml), adjusted to pH 3. At this concentration its buffering capacity is similar to that of our creatinine solution (75 mg/ml), and its effect (243 ± 44, six rabbits) was similar to that of creatinine at pH 3. The scores obtained with ATP in doses of 1 and 2·5 mg/kg were 210 ± 51 and 266 ± 30 respectively.

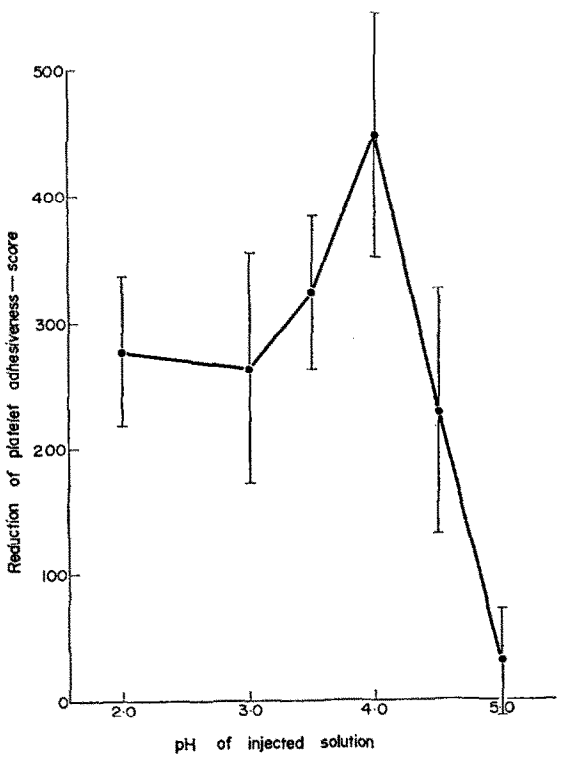


FIG. 2. Effects on platelet adhesiveness of intramuscular injections of creatinine solutions (75 mg/ml/kg) of various pH values. Each point is the mean value for four rabbits ± S.E. Reductions of platelet adhesiveness are indicated by a score that embraces the degree and duration of effect (see text).

We conclude from these experiments that the ability of acidic creatinine or glycine solutions to reduce platelet adhesiveness after intramuscular injection stems entirely from their acidity and high buffering capacity. The reduction in muscle ATP and the increase in plasma ATP suggests that the acid buffer either liberates muscle ATP into the blood stream or liberates some substance from muscle

that in turn releases ATP into plasma from blood cells or vessel walls. In either case the appearance of ATP in the plasma can account for the reduction in platelet adhesiveness.

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# Analytical and pharmacokinetic studies on the optic isomers of oxazepam succinate half-ester

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PREVIOUS studies have shown an accumulation of oxazepam in the brain of mice, but not in rats after diazepam administration.[1] This accumulation is probably related to a longer half-life of oxazepam in mice than in rats.[2,3] However, since oxazepam is currently available as a racemate, it was of interest to investigate the toxic and the anticonvulsant effects of the two optic isomers prepared as succinate half-esters (see Fig. 1).

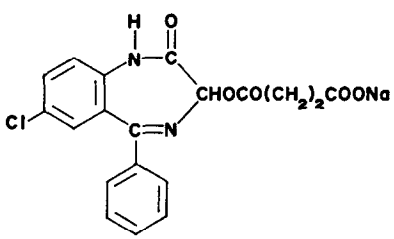


FIG. 1.

In addition the blood levels of oxazepam obtained after the administration of the two isomers or the racemic form of oxazepam succinate half-ester were determined.

*Materials and methods*

(1) *Animals.* Male Sprague–Dawley rats (body weight 200–250 g) and male albino Swiss mice (body weight 20 ± 2 g) fed *ad lib.* were used in all experiments.

(2) *Drug administration.* Oxazepam succinate half-ester (sodium salt) in the two optical isomeric forms (+), (−) and in the racemic form (±)* was administered both intravenously and orally, at a dose of 7·15 mg/kg (corresponding to 5 mg/kg of oxazepam) dissolved in a 0·006 M phosphate buffer at 1 pH 7·38. The $LD_{50}$ was calculated in mice after i.v. administration, according to Lichtfield and Wilcoxon.[4] The anticonvulsant $ED_{50}$ is calculated as the dose in mg/kg i.v. protecting 50 per cent of the mice from the mortality induced by metrazol (120 mg/kg i.p.). The preparation of blood extracts was made according to the method previously described.[5] Gas chromatograph Model G 1(Carlo Erba, Milan) equipped with a $^{63}Ni$ electron capture detector (voltage: 42V). The stationary phase was $OV_{17}$ 3% on Gas Chrom Q (100–120 mesh) packed into a 2-m glass column (int. diam. 2 mm, ext.

* Kindly supplied by U. Ravizza, Muggiò, Milan.