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SHORT COMMUNICATIONS

Effects of 4-prenyl-1,2-diphenyl-3,5-pyrazolidinedione (DA 2370, fenilprenazone*) on lysosomes

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THE EXPERIMENTAL data relative to 4-prenyl-1,2-diphenyl-3,5-pyrazolidinedione (DA 2370, fenilprenazone), a new non-steroidal anti-inflammatory drug, have recently been reported.[1–3] In this paper the effects of this new compound are compared with phenylbutazone, on *in vitro* (liver lysosomal membrane fragility) and *in vivo* (acid phosphatase of kaolin-inflamed rat paws) lysosomal membrane.

According to Ignarro,[4] DA 2370 and phenylbutazone were incubated at 37° for 15 min with aliquots of a suspension with a heavy mitochondrial fraction (3500 g), containing lysosomes in a sucrose–Tris

TABLE 1. EFFECT OF DA 2370 AND PHENYLBUTAZONE ON STABILITY OF LYSOSOMES *in vitro*

Drugs	Concn (M)	Acid phosphatase* (mean ± S.E.)
Incubated		167·30 ± 3·70 (24)
Phenylbutazone	10^{-2}	190·00 ± 3·15† (6)
	10^{-3}	164·69 ± 5·06 (6)
	10^{-4}	138·43 ± 3·70† (6)
	10^{-5}	140·69 ± 4·34† (10)
	10^{-6}	162·34 ± 8·10 (11)
DA 2370	10^{-2}	188·43 ± 3·15† (6)
	10^{-3}	183·30 ± 4·22 (6)
	10^{-4}	140·60 ± 2·79† (6)
	10^{-5}	144·60 ± 3·76† (10)
	10^{-6}	167·21 ± 8·22 (11)

* Aliquots (0·2 ml) of 3500 g of a crude rat liver suspension containing lysosomes were incubated with 2 ml of 0·18 M sucrose–Tris acetate buffer, pH 7·4, with or without added drug for 15 min at 37°. The supernatant fraction separated by high speed centrifugation (27,000 *g* for 15 min at 4°) was used for the assay of acid phosphatase. Data represent mU/mg of nitrogen obtained by the method of Biochemica Test Boehringer 15988. Numbers in parentheses represent experiments.

† Significance relative to incubated samples ($P \leq 0{\cdot}01$ by Student's *t*-test).

‡ The release of acid phosphatase in incubated sample without drugs was 15 per cent of total unsedimentable enzyme activity which was determined by incubation of suspension containing lysosomes in 2 ml of 0·2% Triton X-100 according to the conditions previously described.

§ Data for nonincubated samples without drugs were 101·30 ± 5·67 (25) mU/mg of nitrogen.

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TABLE 2. ACTION OF DA 2370 AND PHENYLBUTAZONE ON ACID PHOSPHATASE OF KAOLIN-INFLAMED RAT PAWS

Treatment	Doses of drugs (mg/kg/orally × times)	Acid phosphatase* (mean ± S.E.)
Controls		11·96 ± 0·61 (10)
Inflamed		18·99 ± 0·37† (10)
Inflamed + phenylbutazone	50 × 2	15·71 ± 0·73†‡ (10)
Inflamed + DA 2370	100 × 2	14·11 ± 0·64‡ (10)

* Values are expressed as milligram of phosphorus/gram of nitrogen. Number of rats in parentheses.

† Significance relative to control group.

‡ Significance relative to kaolin treated group ($P \leqslant 0{\cdot}01$ by Student's *t*-test).

§ Inflammation was induced by a subplantar injection of a 10 per cent suspension of kaolin in water (0·05 ml/paw).

acetate buffer (pH 7·4). After incubation the samples were centrifuged at 27,000 *g* for 15 min at 4° in a MSE high-speed 25 centrifuge and supernatant fractions were assayed for release of acid phosphatase.[5]

Inflammation was induced in male rats of the Sprague–Dawley strain, weighing on average 175 g, by a subplantar injection into both paws of a suspension of 10% kaolin in water (0·05 ml/paw). The two non-steroidal anti-inflammatory drugs were given orally, suspended in 5% acacia gum, in a constant volume of 10 ml/kg and in the doses reported in Table 2, twice daily (the first administration at 9 a.m. and the second administration at 5 p.m.) for one day. Control rats received 10 ml/kg of 5% acacia gum. Twenty-four hr after the injection of the phlogogenic agent, the animals were killed by decapitation, the tissue from the paws was removed and homogenized for the assay of acid phosphatase[6] and for the determination of nitrogen according to Kjeldhal.

The data obtained *in vitro* on lysosomes stability (Table 1) show that both DA 2370 and phenylbutazone in final concentrations of 10^{-4} and 10^{-5} M display a similar action in protecting the lysosomal membrane. These results are in agreement with those of other authors[7–10] referring to anti-inflammatory drugs. Also in our experimental conditions the concentration of the drug was an important factor.[9,10] In fact the two drugs under consideration revealed a biphasic action on lysosomes stabilizing them at relatively low concentrations, but lysing them at higher concentrations.

With regard to the action of local paw concentration of acid phosphatase DA 2370, like phenylbutazone,[11,12] it reduced the increased enzyme in inflamed tissue, also showing an *in vivo* effect in protecting the lysosomal membranes. Local acid phosphatase increased as a consequence of the disruption of lysosomal membranes ascribed to the phlogistic process.

From the literature data[10,12] lysosomal enzymes are generally considered as mediators of the inflammatory processes, but the results concerning the action of anti-inflammatory drugs on the stability of lysosomes are contradictory.[14–16] These contradictions being taken into account, our experimental results reveal the protective action of both DA 2370 and phenylbutazone on lysosomal membranes *in vitro* and *in vivo*.

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Effect of *N*-ethylmaleimide on ($Na^+ + K^+$)-dependent adenosine triphosphatase activity at very low substrate concentration

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N-ETHYLMALEIMIDE (NEM) inhibits ($Na^+ + K^+$)-dependent adenosine triphosphatase [($Na^+ + K^+$)-ATPase] activity[1] without affecting an Na^+-dependent ATP-[^{14}C]ADP transphosphorylation believed to be the initial step of the reaction.[2,3] The authors assumed a sequence of two enzyme-phosphorylated intermediates in the ($Na^+ + K^+$)-dependent reaction, which for the purposes of this communication is simplified as follows:

$$ATP + E_1 \underset{}{\overset{Mg^{2+}\ Na^+}{\rightleftharpoons}} ADP + E_1 \sim P \qquad (1)$$

$$E_1 \sim P \xrightarrow{Mg^{2+}} E_2 - P \qquad (2)$$

$$E_2 - P \xrightarrow{K^+} E + P_i \qquad (3)$$

Several distinctive properties between both phosphorylated intermediates have been described later.[4] It is assumed that NEM inhibits step (2) of the reaction.[2,4]

An Na^+-dependent and K^+-independent ATPase (Na^+-ATPase) activity has been described at very low substrate concentrations in preparations with ($Na^+ + K^+$)-ATPase activity. These two activities were either attributed to different enzymatic sites acting independently,[5] or to a single enzyme or enzyme system.[6]

This paper aims at describing the effect of NEM on Na^+-ATPase activity, in order to provide additional material for the solution of this problem. As yet we have found no report of such study in the literature.

The preparation of rat brain microsomes and the test of Na^+- and ($Na^+ + K^+$)-ATPase activities are reported elsewhere.[6] It was previously shown[6] that the results obtained in the presence of ($Na^+ + K^+$) at low ATP concentrations reveal the ($Na^+ + K^+$)-dependent ATPase activity of the preparation and not a combination of Na^+- and ($Na^+ + K^+$)-dependent ATPase activities. [γ-^{32}P]ATP, prepared according to Glynn and Chapell,[7] was used as substrate. All other reagents were analytical grade. Prior to enzymatic activity tests, the microsomes were preincubated at 37° with different NEM concentrations for different periods.

Two different groups of experiments were performed and typical results are summarized in Table 1.

For ATP concentrations higher than 1 mM, the inhibition of ($Na^+ + K^+$)-ATPase by NEM required preincubation of the enzyme with the drug.[1] The first group of experiments showed that preincubation is also required at concentrations under 1 μM ATP. In addition, the data showed that Na^+-ATPase activity was also inhibited by NEM and that the inhibition of both Na^+- and ($Na^+ + K^+$)-ATPase activities varied similarly with different preincubation periods.