ELEVATION OF RENAL CYCLIC GMP CONCENTRATIONS AND PLASMA LYSOSOMAL ENZYME ACTIVITY FOLLOWING COBALT TREATMENT IN RATS

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SUMMARY

Following an erythropoietic dose of cobalt in rats, elevations in renal guanosine 3',5'-monophosphate (cyclic GMP) concentrations occurred within 10 minutes reaching peak levels within 40 minutes of injection. Subsequent to the cyclic GMP changes, activities of lysosomal enzymes in plasma (β-glucuronidase, acid phosphatase) increased markedly, with maximal elevations of both enzyme activities occurring 2 hours after cobalt treatment. Bilateral nephrectomy abolishes the elevations in plasma lysosomal enzyme activity following cobalt treatment, suggesting that the kidney is the source of these enzymes. It is proposed that a cyclic GMP-mediated release of lysosomal enzymes from the kidney may be an early effect of cobalt leading to the generation of renal erythropoietin.

Goldwasser et al. (1) initially demonstrated that the erythropoietic effect of cobalt resulted from increased kidney production of erythropoietin (ESF), the hormone regulating erythropoiesis (2). The mechanism of cobalt-mediated ESF production has been found to involve a renal enzyme (renal erythropoietic factor, REF, erythrogenin). In vitro studies have suggested that REF interacts with a plasma substrate to generate ESF (3,4). Other studies indicate a lysosomal origin for REF (5,6), as cathepsin activities were correlated with REF activity. Additionally, inhibitors of lysosomal hydrolytic and proteolytic enzymes blocked the in vitro generation of ESF (6). Ignarro and George (7) have linked lysosomal enzyme release to elevations in tissue cyclic GMP concentrations. These investigators postulate that agents which induce lysosomal enzyme release do so by increasing intracellular cyclic GMP

levels, thus subsequently triggering the secretion of lysosome granule contents (8). In the present study, renal concentrations of cyclic GMP and plasma lysosomal enzyme activities were measured in an attempt to determine if a cyclic GMP-mediated lysosomal enzyme release might occur following an erythropoietic stimulus.

MATERIALS AND METHODS

All biochemicals were purchased from Sigma Chemical Company. Male Sprague-Dawley rats (200-250 g) were the source of tissues in these experiments. Renal extracts for measurement of cyclic GMP concentrations were prepared as previously described (9). A radioimmunoassay obtained from Collaborative Research was used to determine renal cyclic GMP concentrations in rats following cobalt treatment (cobaltous chloride hexahydrate - 250 μmoles/kg, s.c.). Purification of tissue extracts proved unnecessary as (1) preparation of purified extracts from columns of Dowex-1 resin and neutral alumina yielded identical cyclic GMP concentrations to that found in unpurified samples and (2) incubation of the unpurified samples with phosphodiesterase abolished the cyclic GMP activity found in non-phosphodiesterase-treated samples.

Acid phosphatase and β-glucuronidase were the lysosomal enzyme markers measured in plasma from another group of cobalt-treated rats. Cobaltous chloride hexahydrate (250 μmoles/kg, s.c.) was administered, and at various time intervals after cobalt treatment, the animals were anesthetized with ether. Blood was obtained from the abdominal aorta in heparinized syringes (100 units/ml), and plasma was prepared by centrifugation of the blood at 2000 x g for 20 minutes. Plasma was stored at -50°C until assayed for lysosomal enzyme activities. Acid phosphatase was measured using p-nitrophenyl phosphate as the substrate (10). The activity of β-glucuronidase was assayed using phenolphthalein glucuronide as the substrate (10). Protein content of the plasma was determined using the method of Lowry *et al.* (11). Lysosomal enzyme activities were expressed as follows: acid phosphatase - μg substrate hydrolyzed/mg protein/15 minutes; β-glucuronidase - ng substrate hydrolyzed/mg protein/hour.

RESULTS

The levels of renal cyclic GMP following cobalt treatment are shown in Figure 1. Control values for cyclic GMP concentrations were 1.48 ± 0.12 x

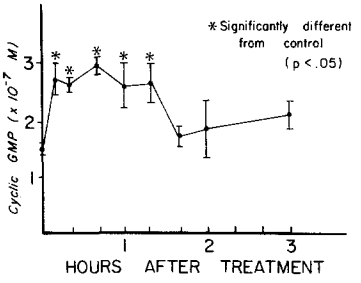

Figure 1. Renal cyclic GMP levels after cobalt treatment. Rats were treated with cobalt, kidneys removed after various intervals and cyclic GMP content determined as described in "Materials and Methods". Each value represents the mean ± standard error of 4 experiments. Asterisks denote a significant difference from control ($p < .05$).

10^{-7}M. As early as 10 minutes after cobalt injection, renal cyclic GMP levels were significantly ($p < .05$) elevated to 2.73 ± 0.31 x 10^{-7}M. Maximal values of 2.93 ± 0.21 x 10^{-7}M were achieved after 40 minutes. Renal cyclic GMP values returned to control levels 100 minutes after cobalt treatment.

Plasma lysosomal enzyme content was next monitored after cobalt treatment. The activities of acid phosphatase and β-glucuronidase are depicted in Figure 2. Control plasma activities of acid phosphatase and β-glucuronidase were 29.3 ± 1.5 μg/mg protein/15 minutes and 46 ± 3.0 ng/mg protein/hour, respectively. The basal values are in excellent agreement with previously reported findings (12). Both enzyme activities were elevated 30 minutes after cobalt injection, although acid phosphatase values were not significantly different from control. At all other time periods studied, the activities of both enzymes were significantly ($p < .05$) greater than control with maximal values occurring at the 2 hour interval (acid phosphatase - 47.8 ± 4.4 μg/mg protein/15 minutes and β-glucuronidase - 248 ± 10.8 ng/mg protein/hour). In separate experiments, cobalt was found not to interfere with the chemical

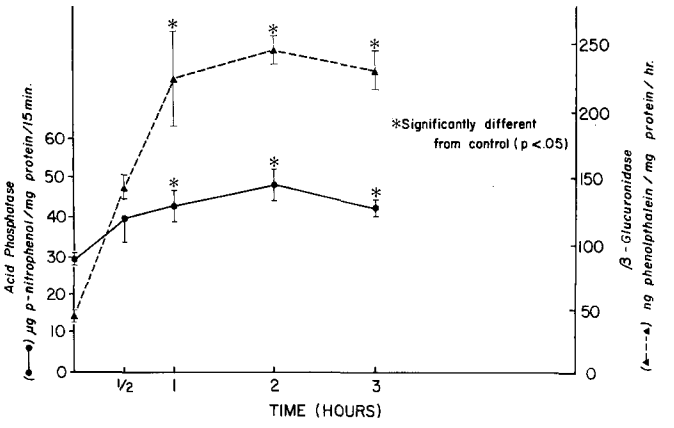

Figure 2. Acid phosphatase and β-glucuronidase activities in plasma following cobalt treatment. Plasma was prepared from animals treated with cobalt at various intervals and assayed for lysosomal enzyme activity described in "Materials and Methods". Each value represents the mean ± standard error of 4 experiments. Asterisks denote a significant difference from control ($p < .05$).

assays for the activities of these lysosomal enzymes. In the present studies, β-glucuronidase may be a more representative lysosomal enzyme marker than acid phosphatase since hydrolysis of p-nitrophenyl phosphate is not totally specific for phosphatase of lysosomal origin (13).

DISCUSSION

These studies demonstrate that following an erythropoietic dose of cobalt, lysosomal enzyme activity in the plasma is increased and is correlated with an increase in renal cyclic GMP concentrations. In an earlier study by Smith et al. (12), elevations in plasma lysosomal enzymes were found 6-24 hours after cobalt treatment. More importantly, these investigators found that bilateral nephrectomy abolished the increases in plasma lysosomal activity. These results (12) combined with those of the present study indicate that cobalt treatment releases lysosomal enzymes into the plasma. The source of these enzymes is probably the kidney and the enzyme release may be preceded by elevations in renal cyclic GMP concentrations. The ability of cyclic GMP to labilize kidney lysosomes and promote enzyme release has already been demonstrated (8).

The erythropoietic enzyme REF has been suggested to be lysosomal in origin (5,6). It might therefore be expected that REF would also be released into the plasma following cobalt treatment; ESF would then be generated by the action of REF on a plasma substrate (3). This hypothesis indicates that renal cyclic GMP may play a role in ESF production via release of REF.

The biological role of cyclic GMP has only recently been investigated. This nucleotide was first implicated in the cholinergic mechanism of acetylcholine (9,14); later studies (15,16) have suggested that cyclic GMP may also be involved in actions such as cellular proliferation and other events as a biological antagonist to adenosine 3',5'-monophosphate (cyclic AMP). One effect of cyclic GMP in hormonal production that may be analogous to the present study is that described by Cehovic et al. (17). These investigators found that cyclic GMP might mediate the release of growth hormone, a process that may be cholinergically-controlled (18).

The mechanism by which renal cyclic GMP concentrations could be increased by cobalt treatment may be related to either enhanced synthesis via renal guanylate cyclase or decreased degradation via renal phosphodiesterase. Preliminary evidence indicates that cobalt does not inhibit renal phosphodiesterase hydrolysis of cyclic GMP. The other possibility is that cobalt acts to elevate renal cyclic GMP levels by either direct or indirect stimulation of renal guanylate cyclase activity.

Previous investigations from our laboratory (19,20) also support the concept of a role for cyclic AMP in ESF production. In these studies, REF which was activated by a cyclic AMP-stimulated protein kinase (20), generated significant amounts of ESF in vitro. Elevations in renal cyclic AMP concentrations and increases in REF activity after cobalt treatment occur much later than the increases in the levels of renal cyclic GMP (Figure 1) and release of lysosomal enzymes (Figure 2). Thus, both renal cyclic GMP and cyclic AMP appear to be involved in the production and/or release of ESF; cyclic GMP may mediate the early lysosomal release of REF, whereas cyclic AMP may participate in a later process of REF activation.

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