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CREATINE KINASE-BB ISOENZYME IN RAT PLASMA AFTER CHRONIC LEAD INTOXICATION

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Creatine kinase (CK) activity in plasma obtained non-invasively from adult healthy, Sprague-Dawley, male rats was found to be 528 ± 270 U/L (N = 17), a value which was 7 times that obtained in human specimens. Agarose gel electrophoresis revealed that the only detectable CK isoenzyme present was CK-BB, in contrast to the human serum isoenzyme which was CK-MM. Furthermore, it was found that the rat CK-BB could be detected using an RIA technique designed to quantitate human CK-BB occasionally present in blood after brain injury (rat CK-BB = 84.5 ± 55.2 μg/L, N = 17, human CK-BB: Not detectable). It was thus possible to calculate the CK-BB specific activity (SA) in rat plasma using total CK assay and RIA (rat CK-BB SA = 6.25 ± 3.87 U/μg, N = 17). When six rats (156 ± 23 g) were treated with lead acetate in the drinking water (26 mM) for 3 weeks, the CK-BB SA rose to 18 ± 5.8 U/μg ($P < .02$). At this point the electrophoresis pattern of the CK-BB showed a transient change from a single band to a doublet. The dose was then increased to 52 mM for 6 weeks, during which time the CK-BB SA declined steadily to 1.6 ± 0.6, a level significantly less than that of the untreated animals ($p < .02$). The results suggest that chronic lead treatment evokes a biphasic response in CK-BB SA with the initial release of enzyme of high SA from tissues. Further treatment apparently results in an inactivation of the enzyme within lead sensitive tissues.

The appearance of the creatine kinase isoenzyme CK-BB in human blood is considered to be an indication of possible brain damage (1), although the intestinal tract and kidney are also rich in this isoenzyme (2,3). Therefore, since lead poisoning can result in disorders involving these organs (4,5), it is possible that CK-BB might be a useful parameter to measure in order to assess the degree of damage to these vital organs. Other workers have suggested that hypoxia induces release of CK-BB from the rat brain (6), but the presence of the isoenzyme in control rat blood has also been shown (7). This study was therefore designed to establish by different methods the content, if any, of CK-BB in healthy rat blood and to investigate any changes which might occur in these levels during lead intoxication. A commercially available radioimmunoassay (RIA) kit specific for CK-BB (8) was utilized to

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calculate the specific activity of the circulating isoenzyme and a correlation was made with the levels of lead within the rat erythrocytes.

METHODS

Male Sprague-Dawley rats (140-170 g) were chosen for this study. Since pentobarbitone has been shown to elevate hamster blood creatine kinase levels (9), samples of blood were collected without anesthesia from the tip of the tail into heparinized tubes. After the plasma was separated by centrifugation at 1000 g and 4°C for 15 min, the erythrocytes were lysed with 3 times their volume of 1 mM digitonin. The hemolyzates were cleared of stroma and debris by a further centrifugation step and stored at -20°C pending lead analysis. The latter was performed by Reference Laboratories Inc., Newberry Park, CA using anodic stripping voltammetry (10). The plasma was maintained below 4°C at all times. Total creatine kinase in the plasma was measured promptly in 10 µL aliquots using a Flexigem centrifugal analyzer (Electro-Nucleonics Inc., Fairfield, NJ 07006), as previously described (11). The isoenzymes of creatine kinase were separated by agarose gel electrophoresis and by ion-exchange chromatography (12). The CK-BB RIA was performed using a kit provided by Mallincrodt Inc. (St. Louis, MO). Since earlier studies by the author (13) and other workers (8) have shown the usefulness of this kit in measuring both the active and inactive forms of CK-BB in human serum, it was validated for rat plasma by a serial dilution technique as follows: Rat plasma containing an apparent CK-BB level of 80 µg/L was serially diluted with the zero standard solution to a theoretical level of 5 µg/L. The actual values obtained by the RIA on these samples compared favourably with the theoretical levels ($r = .991$), indicating that the technique was valid for monitoring CK-BB fluctuations in rat plasma, although it is not yet proven if the method gives an accurate measure of the concentration of the peptide. The presence or absence of fibrinogen in the rat samples did not affect the CK-BB assay by RIA. The effect of lead acetate on the creatine kinase activity was also assessed by adding an aqueous solution of the compound directly to the assay mixture just prior to analysis on the Flexigem. It was found that 10, 20, and 30 µM caused a decrease of 7, 33 and 100% in the estimation of enzyme activity, respectively.

RESULTS

As illustrated in Fig. 1, the Corning control serum contained the 3 isoenzymes of creatine kinase in their expected positions after electrophoresis on agarose gel, with the CK-BB migrating to the most anodal position. The only isoenzyme detected in the untreated rat plasma had the same electrophoretic mobility as CK-BB in the control run. In the samples from treated rats, however, a peak in the CK-MM region (origin) was occasionally seen. In all instances where this occured, the electrophoresis run was repeated and the membranes incubated with the usual CK reagents, but without phosphocreatine to initiate the reaction. Except for the samples in the eighth and ninth week of the experiment (which had some CK-MM), this procedure resulted in the reappearance of the peak at the origin and the

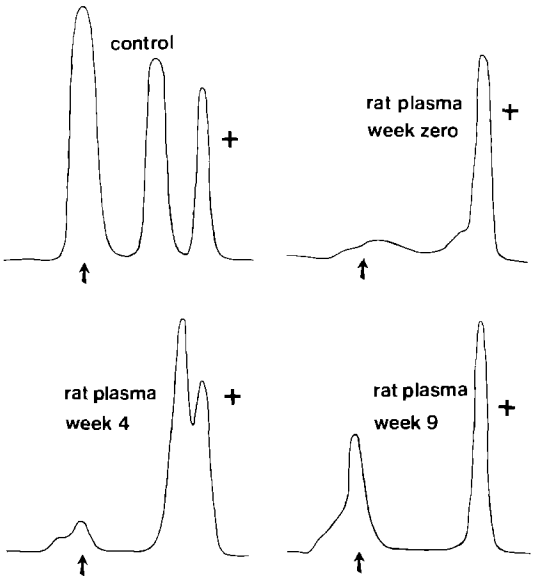

FIGURE 1. Fluorometric scans of CK isoenzymes following electrophoretic separation on agarose gels. The control scan shows the position of the marker isoenzymes, CK-MM, CK-MB and CK-BB. The other three scans were performed with rat plasma obtained at the indicated time after lead treatment was initiated.

absence of a peak in the CK-BB region, suggesting that adenylate kinase accounted for the peak in the CK-MM region. It can also be seen in Fig. 1 that an extra peak in the CK-BB region was present in some of the treated specimens. This CK-BB doublet appeared after 2 weeks and was visible in samples taken up to the fifth week of lead treatment. Thereafter, only the single CK-BB peak was seen.

The total creatine kinase activity was measured in plasma from 17 rats and found to be 528 $\pm$ 270 U/L (mean $\pm$ S.D.). This contrasts to the level found in 17 healthy, male, laboratory persons which was 76 $\pm$ 38 U/L. The CK-BB levels, as determined by RIA, was 84.5 $\pm$ 55.2 µg/L in rat plasma, but was not detectable in human serum. Thus, the CK-BB SA was 6.25 $\pm$ 3.87 U/µg in the untreated rat plasma.

Fig. 2 shows the pattern obtained when the CK-BB SA was monitored over a period of 9 weeks during which lead was administered to the animals by adding lead acetate to the drinking water (distilled) using the following concentrations: 26 mM for 3 weeks and 52 mM for the remaining 6 weeks. As

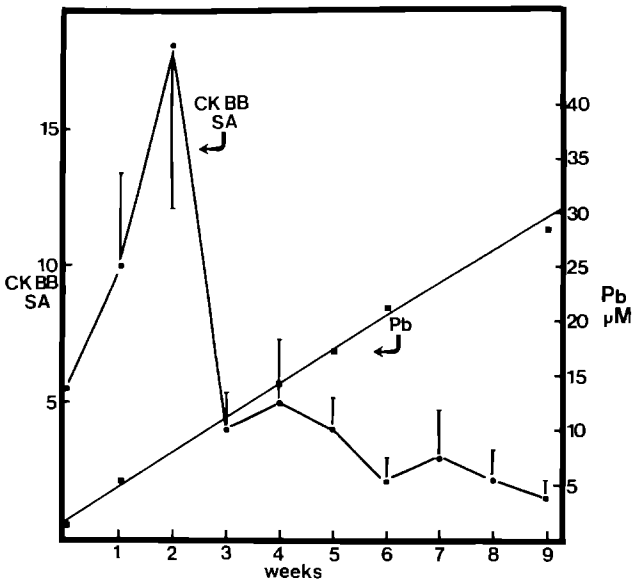

FIGURE 2. The mean CK-BB specific activity in plasma obtained from 6 rats at various times after the initiation of lead treatment (represented by dots). Bars indicate the standard deviation. The mean concentration of lead in whole rat blood is indicated by squares. After 3 weeks of lead treatment at a level of 26 mM in the drinking water, the dosage was doubled to 52 mM.

can be seen in the diagram, the initial treatment with the lead acetate resulted in a 3-fold increase in CK-BB SA after 3 weeks to 18 $\pm$ 5.8 U/μg which was significantly different from the initial level ($p < .02$). Thereafter, it declined steadily until the termination of the experiment after 9 weeks of lead treatment. The mean CK-BB SA at this time was 1.6 $\pm$ 0.6, a level which was significantly lower than for the untreated rats, ($p < .02$). In those instances where CK electrophoresis indicated that CK-MM was present (see above), ion-exchange chromatography was performed on an aliquot of the plasma and the CK-MM activity determined on the appropriate eluate; this was deducted from the total CK activity present in the original sample before the CK-BB SA was calculated.

The mean whole blood lead concentrations at various times during treatment can also be seen in Fig. 2. It will be noted that the levels of blood lead rose steadily throughout the experiments. The mean weight of the 6 rats increased by 29% during the 9 weeks of treatment.

DISCUSSION

In the knowledge of the author, there are no reports in the literature concerning the high total CK activity (7 times the human level) in rat blood, obtained without anesthesia or laparotomy, both of which procedures could affect CK levels (9). It is conceivable that some of the CK activity could derive in vivo from the high concentration of CK-BB found in rat platelets, but not in human platelets (14). Since the enzyme could leak out of the platelets in the clotting process (7), the experiments described here were all performed on plasma samples. Rat lymphocytes are another potential source of plasma CK-BB (15).

Although rat muscle contains CK with the same mobility as that from human muscle (16), the lack of this isoenzyme in the plasma or serum of young rats, reported by Shibata and Kobayashi (7), and confirmed here, suggests that there is a fundamental difference between humans and rats in the manner in which CK leaks from muscle cells into the circulation.

The electrophoretic and RIA evidence of CK-BB activity in healthy rat blood supports earlier reports (7,14), and further suggests that caution should be used in interpreting the presence of the isoenzyme in hypoxia or oleate treatment as necessarily an indication of brain damage (6,17), at least when the rat is used as the experimental model. However, since CK-BB from the rat and human brain is composed of two components (18,19,20), and since an extra CK-BB band has been found in mouse serum after herbicide intoxication (21), the appearance of a doublet in the circulation would seen to be a better indication of brain damage. The finding, reported here, of a doublet in the CK-BB region after 2 weeks of lead treatment, supports the concept that some brain damage was occuring in these animals. The reason why the electrophoretic pattern reverted to that of the untreated rats after the fifth week is unknown, but could be related to an inhibition of the enzyme by a high concentration of lead (30 μM Pb acetate was sufficient to completely inhibit rat CK-BB).

The finding of a three-fold increase in CK-BB SA after 3 weeks of lead treatment could be due to an increased permeability of the cell membranes to

the enzyme as suggested by Gooneratne and Howell (22), who found a transient increase in CK activity after copper treatment. The brain is the most likely source since, (a) a transient release of CK-BB into the circulation has been found after cerebral anoxia (23), and (b) the peak SA coincided with the appearance of the CK-BB doublet on electrophoresis (the brain is the only organ known to contain the doublet, 18,19). Furthermore, the high SA appeared when the whole blood lead concentration was approximately 8 μM, a level which is higher than that associated with lead encephalopathy in children (4,24) or with brain dysfunction in the rat (25). However, since CK-BB is also present in lung, liver, intestine, kidney and bone (2,3,26,27), and since lead is known to accumulate in these tissues (5,28), the plasma CK-BB of low SA found in the latter 4 weeks could have originated from any one of these tissues.

Thus, the presence of a high specific activity CK-BB isoenzyme in the blood might be diagnostic of brain injury in lead poisoning and a CK-BB SA level below normal would suggest more wide-spread organ damage associated with higher blood lead concentrations. CK-BB SA determinations in human specimens, however, would require more sensitive RIA and CK-BB activity determinations than are currently available.

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