血清中クレアチンキナーゼ活性値およびアイソザイムパターンに対する腸管中のクレアチンキナーゼの寄与

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The Contribution of Intestinal Creatine Kinase to Serum Creatine Kinase Activity and Its Isoenzymes in Dogs

Yasushi KIKUTA, and Takafumi ONISHI[1)]

Toneyama Institute for Tuberculosis Research, Osaka City University Medical School, Toneyama, Toyonaka, Osaka 560, and [1)]*Department of Veterinary Medicine, College of Agriculture, University of Osaka Prefecture, Mozuumemachi, Sakai, Osaka 591, Japan*

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ABSTRACT. To investigate the contribution of intestinal creatine kinase (CK) to serum CK activity and its isoenzymes, neostigmine, an anticholinesterase agent, was injected into 8 dogs to stimulate intestinal peristalsis. Serum CK activity was increased 3 hr after injection and had a similar isoenzyme pattern to that of intestine. CK activity decreased to normal range within 24 or 48 hr. Serum CK activities did not change in dogs injected both with neostigmine and stropine, or only atropine. Thus hyperperistalsis of the interstine might increase the activity of serum CK and altere its isoenzyme pattern. Intestinal CK could contribute to serum CK activity and its isoenzyme pattern.—KEY WORDS: creatine kinase, dog, hyperperistalsis, neostigmine.

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Creatine kinase (CK; EC 2.7.3.2) is an important high-energy transfering enzyme abundant in brain, skeletal muscke, heart and smooth muscle [5, 6, 7, 11]. CK consists of three isoenzymes: CK-MM (muscular type), CK-BB (brain type), and CK-MB (hybrid type) [10]. If the integrity of cell membrane is altered, CK leaks from cells [12]. Elevation of serum CK activity has been reported to occur in association with muscle diseases such as myocardial and intestinal infarction in man [2, 9], a stress and malignant hyperthermia syndrome in pigs [4], and rhabdomyolysis (tying-up syndrome) in horses [13].

Serum CK has been the subject of various experimental and clinical investigations in dogs to establish a model for human myocardial and intestinal infarction [3, 9], and to verify that serum CK activity can be used in diagnosing muscular diseases [1]. Although canine smooth muscle has very high CK activity [5, 6, 7], serum CK activity associated with intestinal disorder of dogs has not been critically evaluated.

The following study was designed to evaluate the effect of intestinal hypermotility on serum CK activity and its isoenzymes. Neostigmine, an anticholinesterase agent, was used to cause experimental hyperperistalsis.

MATERIALS AND METHODS

Twenty healthy adult mongrel dogs ranging in weight from 6 to 12 kg were used. They were randomly divided into three groups. In Group A, 8 dogs were injected subcutaneously with a single dose of 0.05 mg/kg neostigmine (neostigminemethyl sulfate, Shionogi & Co., LTD., Osaka, Japan). In Group B, 7 dogs were injected subcutaneously with neostigmine in the same manner in Group A, and also injected subcutaneously with 0.05 mg/kg atropine (atropine sulfate, Fuso Pharmaceutical Industries, LTD., Osaka, Japan) four times; 15 min before, and 45, 105 and 165 min after injection of neostigmine. In Group C, 5 dogs were injected subcutaneously with 0.85% NaCl solution instead of neostigmine, and with atropine as well as Group B. Serum was separated from venous whole blood ob-

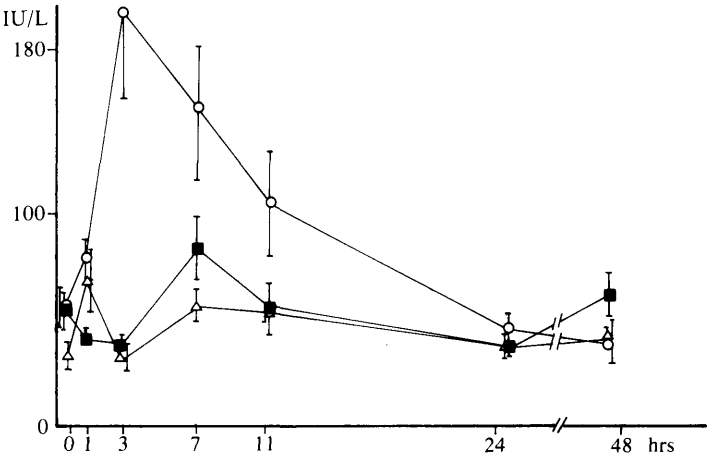

Fig. 1. Serial changes in serum CK activities of each group. Bars represent standard error. o : Group A dogs injected with neostigmine (0.05 mg/kg, subcutaneously) (N=8). ■ : Group B dogs injected with neostigmine (0.05 mg/kg, subcutaneously) and atropine (0.05 mg/kg, subcutaneously) (N=7). △ : Group C dogs injected with atropine (0.05 mg/kg, subcutaneously) (N=5).

tained just before injection of neostigmine or 0.85% NaCl solution, and 15 min, 1, 3, 7, 11, 24 and 48 hr after injection.

Serum CK activities were measured with the aid of an autoanalyzer (CPK UV-AR; WAKO Chemical Industry, LTD., Osaka, Japan, and HITACHI 712 Autoanalyser; Hitachi, LTD., Tokyo, Japan) using Rosalki's method [8]. Electrophoretic separation serum CK isoenzymes was performed (300 V, for 15 minute), using a color reagent (CK Isoenzyme Reagent; Helena Lab., U.S.A.), cellulose acetate membrane (Titan-III Iso Flur, Helena Lab., U.S.A.), and Tris-barbital buffer (pH=8.6; μ=0.03). Densitomerter, Densitron Model PAN-FV (Jookoo Co, LTD., Tokyo, Japan), was used to scan serum CK isoenzymes.

RESULTS

Group A dogs,injected with neostigmine, developed into variable degrees of abdominal pain, vomiting and diarrhea about 15 min after injection with neostigmine. These clinical signs disappeared within 24 hr after injection. Three of Group B dogs, injected with neostigmine and atropine, showed vomiting, but the other dogs showed platycoria alone. Dogs injected with atropine, Group C, only developed platycoria.

In Group A dogs, the activity of each serum isoenzyme was significantly increased ($p<0.05$) compared to preinjection values, and that of Groups B and C (Fig. 1, 2, 3, 4). The peak of increased CK in Group A was observed at 3 hr after injection; it progressively decreased to normal within 24 or 48 hr. The mean values of each serum CK isoenzyme in group A dogs at 3 hr after injection were 25.4% (CK-MM), 33.1% (CK-MB), 41.1% (CK-BB) of increased serum CK activity, deducted activity before injection from that after injection.

DISCUSSION

Neostigmine is an anticholinesterase agent that influences the parasympathetic and motor nervous systems. Atropine is an anticholinergic agent that can completely inhibit the effect of neostigmine on the parasympathetic

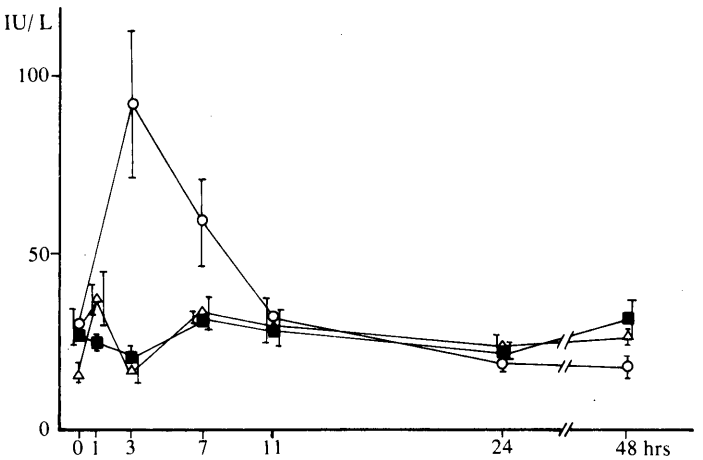

Fig. 2. Serial changes in serum CK-MM activities of each group. Bars represent standard error. ○: Group A dogs injected with neostigmine (0.05 mg/kg, subcutaneously) (N=8). ■: Group B dogs injected with neostigmine (0.05 mg/kg, subcutaneously) and atropine (0.05 mg/kg, subcutaneously) (N=7). △: Group C dogs injected with atropine (0.05 mg/kg, subcutaneously) (N=5).

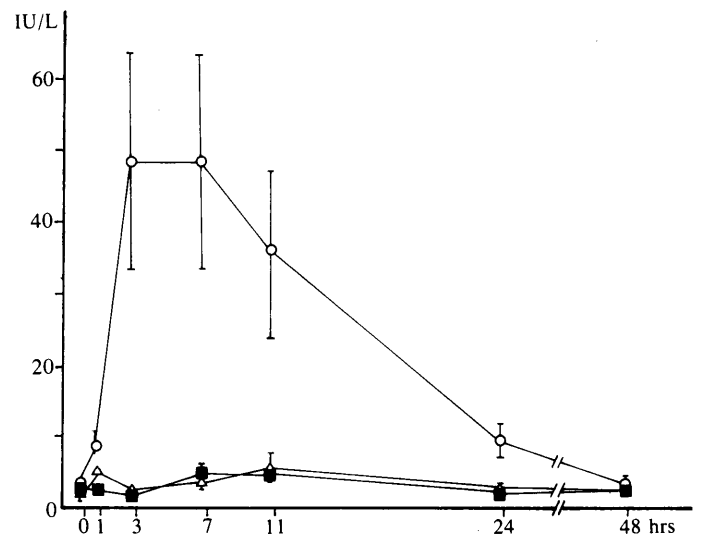

Fig. 3. Serial changes in serum CK-MB activities of each group. Bars represent standard error. ○: Group A dogs injected with neostigmine (0.05 mg/kg, subcutaneously) (N=8). ■: Group B dogs injected with neostigmine (0.05 mg/kg, subcutaneously) and atropine (0.05 mg/kg, subcutaneously) (N=7). △: Group C dogs injected with atropine (0.05 mg/kg, subcutaneously) (N=5).

systems. In Group A dogs, neostigmine would affect both the parasymathetic and motor nervous systems. In Group B dogs, neostigmine would affect only the motor nervous systems, because atropine inhibited the parasympathetic systems. In Group C dogs, atropine affected only the parasympathetic systems. Therefore hyperperistalsis could oc-

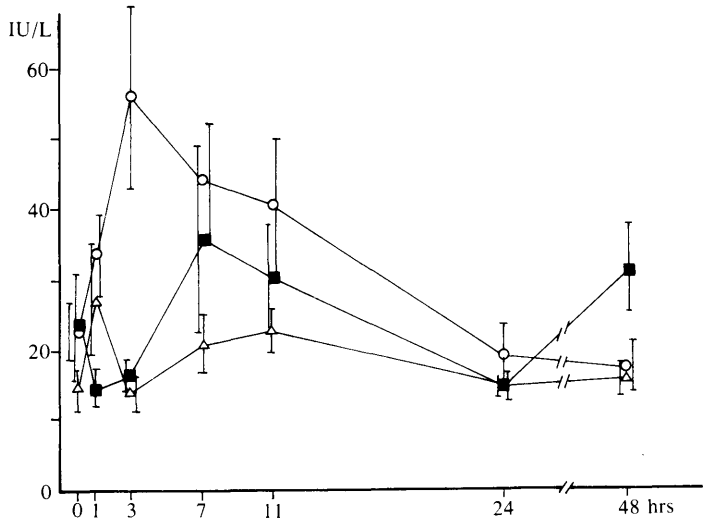

Fig. 4. Serial changes in serum CK-BB activities of each group. Bars represent standard error. ○ : Group A dogs injected with neostigmine (0.05 mg/kg, subcutaneously) (N=8). ■ : Group B dogs injected with neostigmine (0.05 mg/kg, subcutaneously) and atropine (0.05 mg/kg, subcutaneously) (N=7). △ : Group C dogs injected with atropine (0.05 mg/kg, subcutaneously) (N=5).

cure only in Group A dogs. Although neostigmine has the potential to influence CK activity of the heart, arterial walls and possibly other tissues, these effect did not detectably increase serum CK activity in this experiment.

In our previous data, canine intestine had high CK activity (about 100 IU/g), and the value was about one nineth of the activity of skeletal muscke and three quaters of that of brain [6]. The isoenzyme pattern of intestine was 12.2% (CK-MM), 34.7% (CK-MB), 53.1% (CK-BB) [6]. In this experiment, serum CK activity was exclusively elevated in Group A dogs, and there was a similarity between the increased CK isoenzyme pattern of serum 3 hr after injection and intestinal CK. Thus neostigmine induced-intestinal hyperperistalsis apparently caused the elevation of the activities of serum CK isoenzymes.

The source of serum CK activity increased transiently in Group A was thought to leak out of cell membrane altered permeability without cell damage in the intestinal smooth muscle. Same findings were observed in blood CK-MM activity after exercise in dogs or horses [12, 13]. But in ischemic experiment elevation of serum CK activity became much higher than this experiment and did not return to normal range within two days, and its isoenzyme pattern was not similar to that of intestinal CK [3]. CK must leak from not only intestine but also other tissues on ischemic experiment.

Results of this study suggest that intestinal hyperperistalsis of the intestine induced by other causes may elevate serum CK activity having similar isoenzyme pattern to the smooth muscle. Furthermore, smooth muscle may be the major tissues that provide CK-MB and CK-BB for serum.

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要　約

血清中クレアチンキナーゼ活性値およびアイソザイムパターンに対する腸管中にクレアチンキナーゼの寄与：菊田安至・大西堂文[1)]（大阪市立大学医学部付属刀根山結核研究所，[1)]大阪府立大学農学部獣医学科家畜内科学教室）――血清中のアレアチンキナーゼ（CK）およびそのアイソザイムに対する腸管中のCKの影響を調べるために，ネオスチグミンを8頭の犬に投与して腸管の蠕動を亢進させた。血清中のCK活性値は投与後3時間目まで増加を示し，腸管のアイソザイムパターンと似たパターンを示した。このCK活性の上昇は投与後24～48時間でみられなくなった。ネオスチグミンとアトロピンの両方を投与した群およびアトロピンだけを投与した群では血清中のCKに変化はみられなかった。このように腸管の過蠕動により血清中のCK活性およびアイソザイムパターンに変化がみられ，腸管中のCKが血清中のCKに影響を与えることがわかった。