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Electromyographical Studies on Experimental Hypocalcemia in Goats

Chung Boo Kang, D.V.M., M.S., Ph.D.

Department of Veterinary Medicine, Faculty of Agriculture, University of Tokyo

Introduction

Since Little and Wright[15] apparently were the first investigators to publish data revealing hypocalcemia in cows showing clinical milk fever, numerous papers as to the etiology including the endocrinical, metabolic, and calcium kinetics studies of hypocalcemia have been done.[1,4,6,7,9,11,14,16-21,25,26,29]

Many papers have been published to report electromyographic studies on the disease of nervous system and muscles.[2,3,8,27] However, no electromyographical studies have been done on hypocalcemia in spite of the frequent occurrence of the disease in some animals.

It is the purpose of this experiment to describe the changes in electromyographic patterns and to serve as the first step to evaluate the mechanism of clinical disturbance accompanied with hypocalcemia considering the electromyographic point of view.

Methods for electromyogram (EMG) recording are commonly conducted in two ways: one is action potential of neuromuscular unit (NMU) and the other is action potential of simultaneous multipe NMU activity. The former is adequate for the analysis in individual motor neuron and muscular function, and the latter is adequate for analysis of intra-muscle activity among the extensors and flexors. Thus, the latter was used in this experiment.

Dairy cattle is desirable as experimental animal, but it is not readily controlled and accurate EMG cannot be recorded accurately from the one tested, thus the goat was used.

Preliminary experiment employing various concentration of EDTA showed that 4% EDTA group goats belonging to the signs of typical hypocalcemia which occurred in 240 minutes of an intravenous infusion and also to evaluate the body response to the more rapid falling of plasma calci-um, 8% EDTA infusion was performed.

Materials and methods

Animals: Five adult healthy Japanese goats weighing 14 to 20kg which were housed in the pasture of university of Tokyo were used. The goats were divided into the three groups. Each 2 of which were used 4% EDTA and 8% EDTA groups. The other one was used for an epidural anesthesia. They were fed conventionally on hay and concentrates in the morning and in the evening. Their plasma calcium concentration were 9.1 to 9.8mg per 100ml.

Infusion of Na_2 EDTA Solution: Disodium ED TA was prepared by dissolving in 5% glucose solution as to 4% EDTA and 8% EDTA solution. The infusion rate was intravenously at one ml per minute and was continued to the occurrence of a cardiac arrest. At any time, about 3ml of blood was withdrawn principally at 15-minute on 4% EDTA and 5-minute intervals on 8% EDTA groups throughtout the experiment.

Blood sample was collected into the heparanized test tube and centrifuged. Packed cell volume PCV was done using standard microcapillary tube test. The plasma was used for determination of total calcium (chelate Ca + nonchelate Ca) by an atomic absorption spectrophotometry (AAS) using Trudeau and Freier method[30] with Hitachi 208 type atomic spectrophotometer and nonchelate Ca (plasma Ca) was done by chelometric reaction using OCPC me-

thod[5,10] with Hitachi 139 type spectrophotometer.

Epidural Anesthesia: Five per cent hydrochloride-procaine solution was inserted between the lumber and sacric epidural area to make hind limbs paresis.

EMG Recording: EMG was recorded from each left and right M. gastrocnemius, M. tibialis cranialis, M. rectus femoris and M. semitendinosus in hind limbs of each goat. It was simultaneously recorded by a nine channel electroencephalograph of San-ei instrument Co. model EG-900 type which was equipped with an ink-writing recording system. Electrodes made of a enamel-coated copper wire 120μm in diameter were put into the middle of each muscle. The distance bewteen the electrodes was 1cm. The time constant in recording was 0.001 second.

During the experiment the reflexes (light, corneal, eyelid and anal), body tempearature, pulse, respiration, and the other clinical findings were checked in detail.

Results

Physical Signs: Results from the continuous infusion of EDTA solution were as follows: restless and unsteady behaviors were first observed, and then progressive depression of reflexes and body temperature, a marked unstable standing posture were developed. Furthermore, proceeding to the EDTA infusion ataxia by staggering, reeling, and falling were observed. Attempts to rise became progressively more awkwardly, then failure and finally in some cases systemic convulsion-like signs were observed until to died. These were commonly observed on 4% EDTA and 8% EDTA groups. However, these phenomena were not so evident in 8% EDTA groups compared with 4% EDTA groups.

On 4% EDTA solution infusion, generally, when plasma calcium concentration decreased to about 6mg per 100ml, signs of hypocalcemia began to appear. During induced hypocalcemia, reflexes and body temperature were progressively depressed. The goat was evidently unsteady and slightlly stiff and occasionally showed some muscle tremors and respiration became frequent and heavy.

When plasma calcium concentration was about 5mg or less, the goat repeated sit up and down awkwardly. At about 3mg per 100ml, they failed to rise in spite of the attempts to do so. Reflexes were almost absent in this stage except for anal. And then finally reached to cardiac arrest when plasma calcium concentration of about 1mg per 100ml. The pupils were dilated and unresponsive to light, body temperature fell down from about 39°C of before infusion of EDTA solution to about 37.1°C or 37.2°C.

On the other hand, on 8% EDTA solution infusion signs of hypocalcemia were observed much earlier in time and calcium concentration was a little higher than 4% EDTA infusion groups. For instans, restless and unsteady began to appear when plasma calcium concentration was about 7mg per 100ml, within 10 minutes after the infusion. However, the depression of reflexes and body temperature were not evident. Failure to rise was observed at about 4mg per 100ml, within 20 minutes after infusion and then cardiac arrest was observed within 40 minutes.

Changes in Electromyogram: On the normal standing posture tonic discharge was observed in extensor muscles before infusion of EDTA solution, but flexors were absent the muscle activity (Fig. 1,2). However, the muscle activity changed the patterns according to the decrease of plasma calcium concentration.

When plasma calcium decreased to 6mg per 100ml or below the tonic discharge of some muscles increased with simultaneous phasic discharge, and finally changed to convulsion. In the state of paresis plasma calcium of about 3mg per 100ml or below, the phasic discharge was intermittently observed on both sides of M. tibialis creanialis and M. semitendinosus and right M. gastrocnemius except for M. rectus femoris which showed the tonic discharge until to died. It means that the clonic convulsion was transferred from the tonic convulsion.

This pattern was simultaneously observed and disappeared while it is repeated (Fig. 1.). The same phenomenon in goat No. 1 was admitted to goat No. 2, although it was different plasma calcium

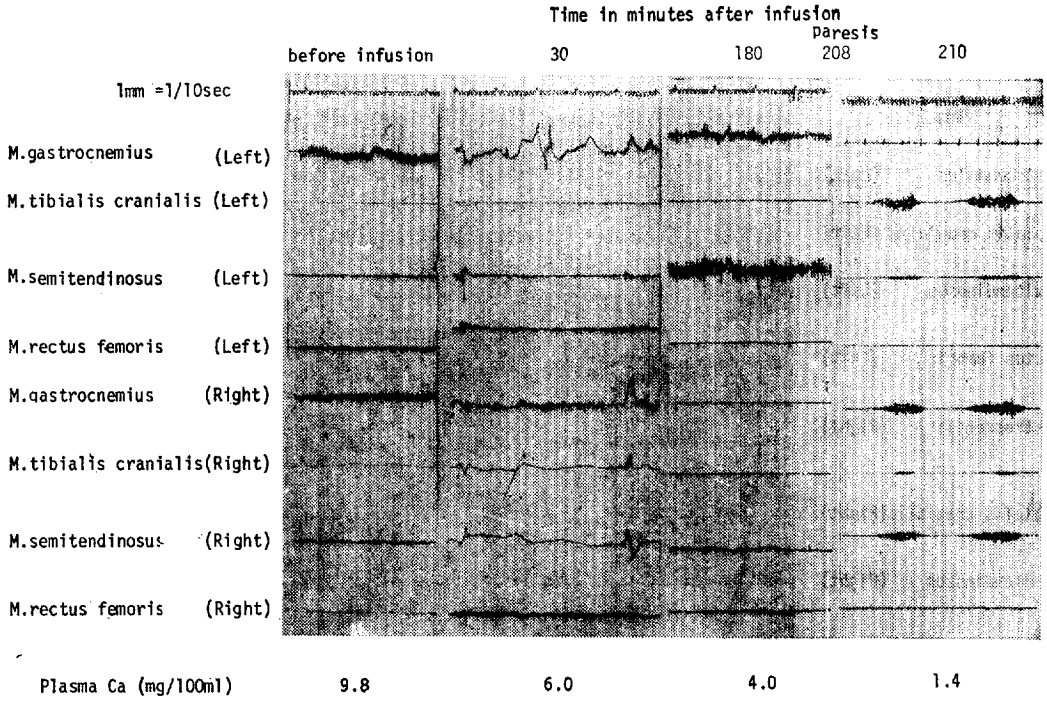

Fig. 1 Electromyograms during continuous infusion of 4% EDTA solution (Goat No. 1).

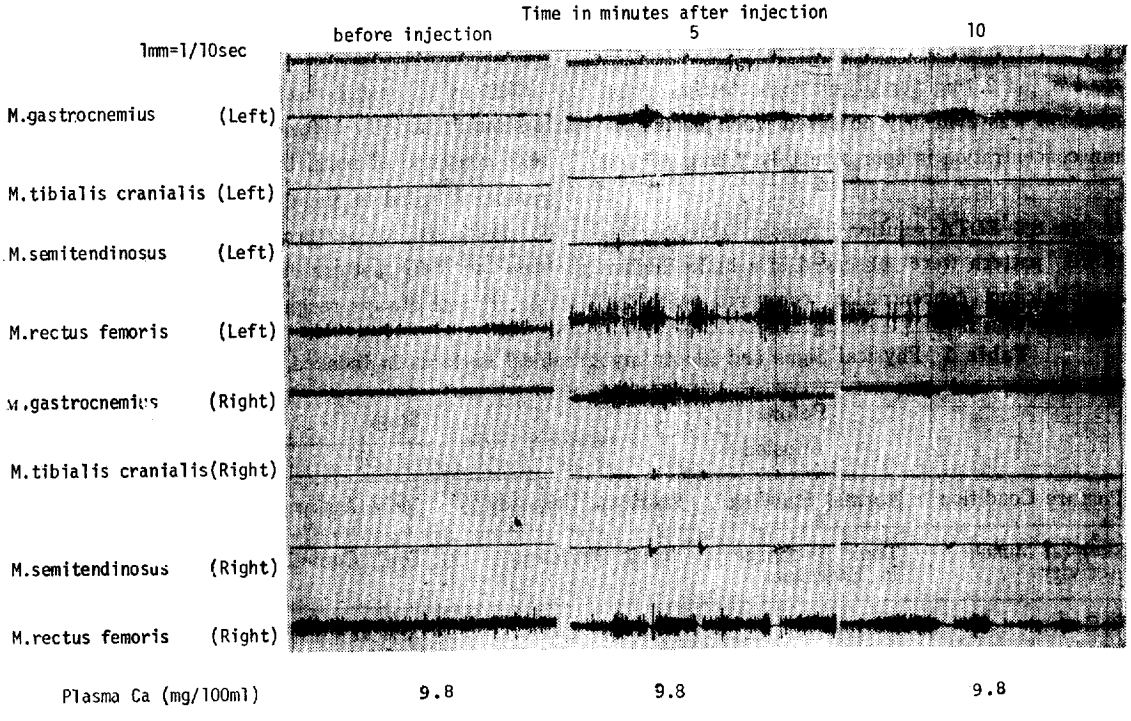

Fig. 2-1 Electromyograms in epidural anesthesia (Goat No. 5).

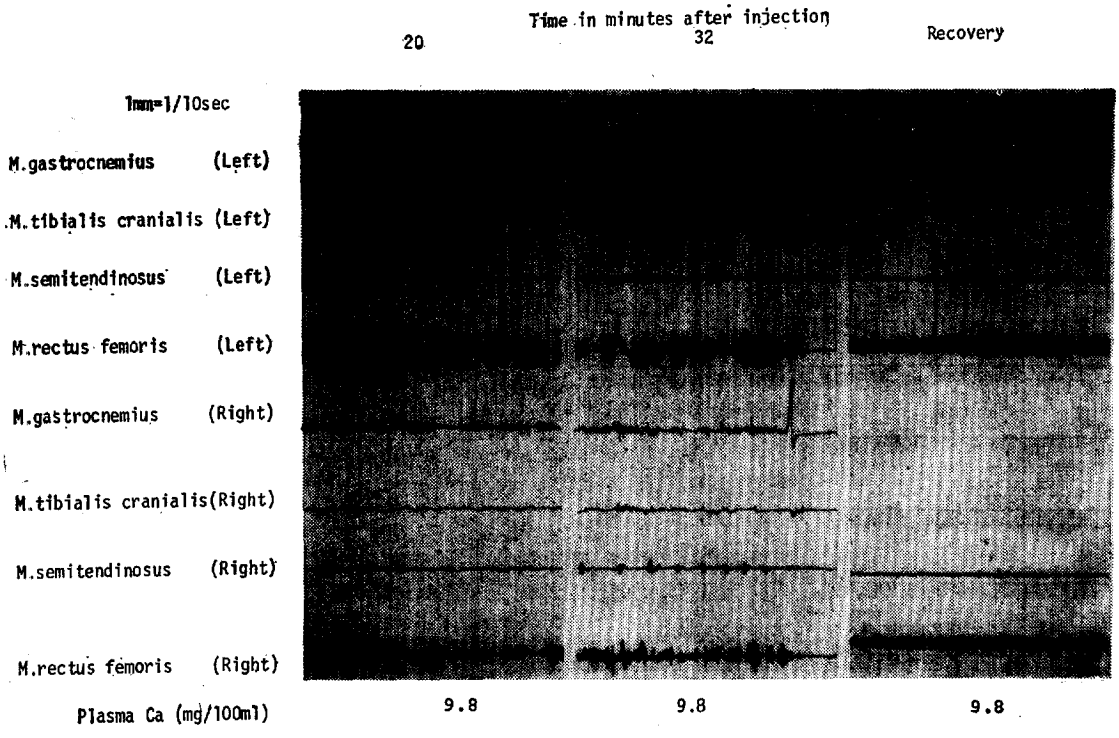

Fig. 2-2 Electromyograms in epidural anesthesia (Goat No. 5).

concentration. In goat No. 2 tonic discharge accompanied with phasic discharge and clonic convulsion were 4.8 and 2.1mg per 100ml, respectively. Tonic convulsion was evidently observed when plasma calcium concentration is approximately 2.8mg per 100 ml.

On the 8% EDTA solution infusion, such changes of EMG pattern were observed in a little higher plasma calcium concentration compared with 4% EDTA groups, but the disorder of reciprocal mechanism was not evident.

EMG by an epidural anesthesia was shown Fig. 2, the muscle activity before the application of anesthesia consisted of normal condition before infusion groups. A trend to anesthesia phasic discharge was observed, but its pattern was evidently different from the EMG pattern in experimental hypocalcemia. It was always reciprocalled between the ext-

Table 1 Physical Signs and Electromyographical Patterns in Induced Hypocalcemia

	Before Infusion	Stage I	Stage II	Stage III
Posture Condition	Normal Standing	Restless, Unsteady	More Awkward	Fail to Rise
Skeletal Muscle Activity	Tonic Discharge in Extensors	Phasic Discharge	Tonic Convulsion	Clonic Convulsion
Reflexes	Normal	Depress	Loss of Cerebral Nerve Reflexes	Loss of Spinal Reflex
Plasma Ca (mg/100ml)	10—9	6—5	4—3	3—0

ensors and flexors at any time. When falling down by hind limbs paresis, its pattern was also reciprocalled. No any muscle activity was observed in lying position, and after recovering from anesthesia the muscle activity was restored to normal one. Physical signs and electromyographical patterns during the experiment are summarized in table 1.

Discussion

As previously described, the typical signs of hypocalcemia were observed both 4% and 8% EDTA solution infusion groups. Especially, the signs began to appear when plasma calcium about 6mg per 100 ml and 3mg per 100ml or less, failure to rise (paresis) was observed in all of 4% EDTA groups.

These results agreed with the plasma calcium concentration of hypocalcemia or experimental hypocalcemia and milk fever in cattle.[9,14,15,23,24,28]

Normal standing posture in the quadrupedal animal mas fixed with knee and tarsal joint, and EMG pattern between the M. extensors and M. flexors in normal standing or movement is reciprocalled so as to make up coordinate regulation of normal posture.[12,13,22] From the EMG pattern this phenomenon was evidently observed. However, the muscle activity changed the patterns according to the decrease of plasma calcium concentration in all of EDTA infusion groups.

Phasic discharge which accompanied tonic discharge was almost simultaneously observed among the M. extensors and M. flexors when plasma calcium about 6mg per 100ml. This pattern is suggested as to abnormal EMG pattern which is suspected the dysfunction in the central nervous system.[8,27,31]

At falling down condition the muscle activity changed to the intermittent phasic discharge which is consist of clonic convulsion. It was almost simultaneously observed and disappeared being of repeating. From this fact, it is not considered a spontaneous muscle activity itself.

On contrast, EMG by an epidural anesthesia proceeding to the anesthesia phasic discharge itself was admitted to the EDTA solution infusion groups. but it was always reciprocalled at any time. In lying position of the hind limbs paresis by epidural anesthesia there was not any muscle activity and there never been observed disorder of reciprocal mechanism and convulsion.

From the mentioned above, EMG patterns during the hypocalcemia were evidently differentiated to an epidural anesthesia. From the physical signs in the EDTA infusion groups, Depression or absence of light, eyelid and corneal reflexes were observed prior to anal reflex. Anal reflex was responsive until to died. From these facts, the appearance of abnormal EMG patterns during the hypocalcemia was considered to be concerned with the dysfunction of central nervous system rather than that of the peripheral nervous system and muscle itself.

On EMG, disorder of reciprocal mechanism was not evident in 8% these groups. It means to investigate the detailed clinical disturbance of hypocalcemia, 8% EDTA solution infusion are not proper for the purpose of it.

Bowen et al.[2] described in parturient paresis of cows resulting from the examination of muscle contractions induced by electrical stimulation of peroneal nerve, indicated that the primary cause of hypocalcemic paresis was depression of neuromuscular transmission.

As described above, from hypocalcemia to failure to rise and cardiac arrest may be possible due to the dysfunction of central nervous system rather than that of peripheral nervous system or neuromuscular transmission and muscle itself. Furthermore, to study the mechanism of clinical disturbance (paresis) of hypocalcemia much more information are required to fully understand it.

Conclusion

Experimental hypocalcemia in healthy Japanese goats was induced by the intravenous infusion of Na_2-EDTA solution of 4% and 8%, respectively. The signs of hypocalcemia were changed according to the decrease of plasma calcium in a study of all of 4 goats.

On electromyogram (EMG) tonic discharge in extensor muscles was observed the normal standing

posture before infusion of EDTA solution. However, when plasma calcium of about 6mg per 100ml. employed the tonic discharge of some muscles increased simultaneously with phasic discharge, and finally changed to the clonic convulsion.

EMG by an epidural anesthesia was always reciprocalled during the experiment at any time. In lying postion by hind limbs paresis, the muscle activity was absent. Therefere, EMG patterns during the hypocalcemia were evidently differentiated to an epidural anesthesia.

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山羊의 低Ca血症에 관한 筋電圖學的硏究

姜 正 夫

東京大學 農學部 家畜內科學敎室

抄 錄

低Ca血症의 主徵인 起立不能의 發現原因의 추구를 위해 實驗的 低 Ca血症에서 起立不能 나아가 心臟運動停止에 이르기까지의 과정을 筋電圖學的으로 실시한 결과의 내용을 요약하면 다음과 같다.

EDTA 注入前의 正常起立時에는 抗重力筋에만 tonic discharge가 얻어졌으나 血中 Ca 濃度의 저하정도에 따라 筋放電이 달라져 6mg/100ml 전후에서 抗重力筋 및 그의 拮抗筋에도 phasic discharge를 수반한 tonic discharge가 同期的으로 나타나 이의 현상은 後軀脫力에 의해 주저앉을 때에도 마찬가지였다. 起立不能의 상태에선 clonic convulsion樣의 筋放電이 폐사시까지 계속되었다.

硬膜外痲醉에 의한 筋電圖에선 筋放電은 항시 相反的이었고 後軀痲痺에 의한 橫臥位 상태에선 어떤 筋放電도 얻어지지 않아 EDTA 注入에 의한 低 Ca血症時와 硬膜外痲醉時의 起立不能의 本質은 相違한 것으로 판명되었다. 또한 低Ca血症時에 수반되는 각종 반사 및 임상증상의 推移로 低Ca血症時에 수반되는 기립불능은 말초신경계 및 神經一筋接合部 및 筋 자체의 기능저하보다는 중추신경계의 기능저하에 의한 가능성을 크게 시사하는 것으로 보아진다.