nMoles of Ca^{++} bound per mg of mitochondrial protein per 200 msec, at $5 \times 10^{-7} M$ Ca^{++}. Estimates of the total mitochondrial content of cardiac muscle[9] indicate values of 30–40 mg of protein. As many as 60 nMoles of Ca^{++} could thus be removed by mitochondria from troponin in 200 msec at room temperature, and this limit would certainly be higher at 37 °C. The rate of Ca^{++} uptake by mitochondria is thus adequate to explain relaxation.

Riassunto. E' stata studiata l'affinità dei mitocondri di fegato di ratto e di cuore di coniglio per il Ca^{++}, nel sistema dipendente da energia. Si sono usati tamponi EGTA-Ca^{++} per mantenere la concentrazione del Ca^{++} costante durante l'esperimento. Si è dimostrato che la K_m apparente per il Ca^{++} non è lontana da $10^{-6} M$.

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Muscle Afferent Outflow During Ethanol Intoxication

KUCERA and SMITH[1] have reported that the addition of ethanol into a Krebs' solution bath surrounding the rat caudal muscle in situ caused a concentration-dependent increase in the activity of afferent nerves from sensory endings in the muscle spindles and tendon organs. The authors suggested that alterations in muscle afferent outflow might be involved in the motor incoordination of ethanol intoxication.

The aim of the present investigation was to examine whether disturbances in the limb muscle spindle afferent function are encountered in vivo following systemic administration of an intoxicating dose of ethanol.

Materials and methods. Male Wistar rats, 250–300 g body weight, were anesthetized by a commercial (Ciba) mixture of allobarbital (8 mg/100 g), urethane (32 mg/100 g) and monoethyl urea (32 mg/100 g) injected i.p. After cannulation of the trachea, a laminectomy exposed the spinal cord from segment L_3 to S_1. A pool containing the exposed spinal cord was filled with mineral oil at 36°C, using the technique described by GLADDEN and KIDD[2]. A hind limb was denervated except for the nerves to the gastrocnemius-soleus complex; the muscles could be stretched to various degrees by weights hung from the toes. The dorsal root L_5 was teased until afferent discharges were obtained from a single muscle spindle stretch receptor. Only afferents exhibiting a sustained discharge in response to loading the Achilles tendon were considered.

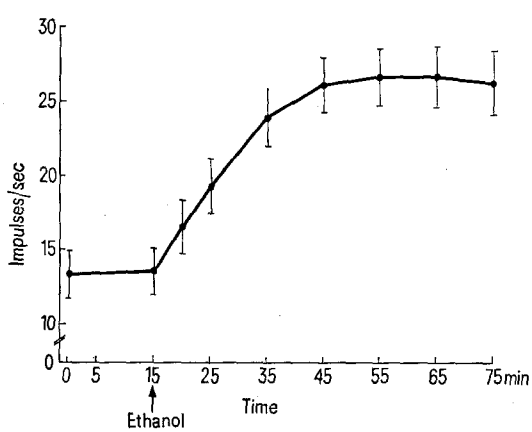

Fig. 1. The effect of i.p. injection of ethanol (2.5 g/kg) on the frequency of firing of 15 muscle spindle afferents. Ordinate: mean frequency of discharge in impulses/sec; brackets encompass ± 1 standard error of mean. Abscissa: time in min.

The afferent activity was measured as described by KUCERA and SMITH[1]. The mean carotid artery pressure was recorded throughout all experiments, using a Statham pressure transducer connected to a polygraph.

Ethanol (Gold Shield absolute alcohol) was given by i.p. injection as a 15% (w/v) solution in isotonic saline in volumes required to provide a dose of 2.5 g/kg body wt. In 3 control experiments, the animals were injected with 1.7 ml/100 g body wt. of a 0.9% sodium chloride solution.

In a parallel study, 15 rats anesthetized as described above were used to determine the rate of appearance of ethanol in blood after the i.p. administration. A cannula was inserted into the central end of the femoral vein and heparin (500 IU/kg) was given. Venous blood samples (0.3 ml) were collected 10, 30, and 60 min after the i.p. injection of ethanol (2.5 g/kg). The plasma samples were analyzed for ethanol content using a Perkin Elmer Model F-11 gas chromatograph fitted with a hydrogen flame ionization detector and a 7% Hallcomid on Porapak Q column.

Results and discussion. The effects of ethanol were studied using 15 muscle spindle afferent fibers from acutely de-efferented muscles. Routinely, the muscle was stretched to obtain an initial discharge rate of 5–20 imp/sec. The i.p. injection of ethanol provoked an acceleration in the activity of afferent nerves originating in muscle spindles; a decrease in the discharge rate was never observed. A noticeable rise in the rate of firing appeared typically as soon as 5 min after the ethanol injection; the firing rate approximately doubled within 60 min. The acceleration was most pronounced within the first 30 min; the response then leveled off and the sensory activity remained fairly constant for the next 30 min. However, the effects of ethanol were not followed longer than 60 min. Figure 1 illustrates the mean time course for excitation by ethanol of 15 spindle afferents; Figure 2 presents oscillo-

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[2] M. H. GLADDEN and G. L. KIDD, J. appl. Physiol. *26*, 501 (1969).

graphic records of the sensory activity in dorsal root L_5 filament in an experiment where the plasma ethanol level reached 340 mg/100 ml at 60 min. In control experiments, the injection of physiological saline did not, by itself, cause any consistent changes in sensory nerve activity.

Shortly after the ethanol administration the mean arterial blood pressure exhibited a variable increase, possibly due to the expansion in the circulatory fluid volume. Thereafter the blood pressure tended to decrease slowly in the course of the 60 min experiment. This pressure drop of 10–30 mm Hg could be halted by allowing the rat to breathe an oxygen enriched air, which was done in about half of the experiments. All experiments in which the pressure decreased below 80 mm Hg were discarded. There appeared to be no correlation between alteration of the blood pressure per se and the afferent activity changes. However, possible significant shifts in regional blood flows, if any, would go undetected. Likewise, other factors, such as adrenal catecholamine release[3] or tissue electrolyte alterations[4] might have influenced the spindle behavior in the course of the experiment.

Published data on the time of peak plasma ethanol level after its i.p. administration vary considerably[3,5,6]; in our experiments, serial blood sampling for the gas chromatographic ethanol analysis revealed that the plasma ethanol level reached its average peak of about 350 mg/100 ml within 10 min after the i.p. injection and declined slightly over the next 50 min (Figure 3). Thus, it would appear that the mean time course of the post-injection spindle excitatory effect is delayed relative to the average plasma ethanol curve obtained in comparable preparations. This delay might reflect the time taken for equilibration of the agent with the extravascular tissue[7] of the spindle assuming that the excitation is due to a direct effect of ethanol on the sensory receptors.

The administration of ethanol in the dose of 2.5 g/kg i.p. to unanesthetized rats induces, among other signs of ethanol intoxication, an impairment of motor function as manifested by decreased performance of the rat in the tilted plane test; the maximal impairment of muscle coordination was seen 15–30 min after the i.p. administration of ethanol[6,8,9].

As was pointed out in the earlier paper[1], disturbances in muscle spindle afferent function such as those observed in the present experiments could be anticipated to participate in the deteriorative action of ethanol on the motor function in intoxicated animals. However, the effect of ethanol upon the muscle spindle under fusimotor control as well as the contributions of actions of ethanol on the afferent systems, relative to its effects centrally, are yet to be determined.

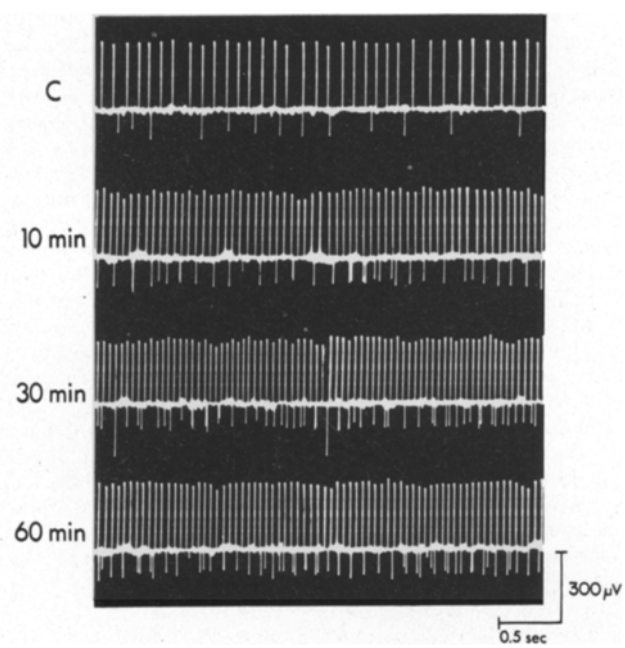

Fig. 2. Records of afferent activity in dorsal root L_5 filament before (C) and at 10, 30 and 60 min after i.p. injection of ethanol (2.5 g/kg). The large up-going unit was identified as originating in a muscle spindle. Plasma ethanol level at 60 min reached 340 mg/100 ml.

Zusammenfassung. Es wird nachgewiesen, dass bei Alkoholintoxikation eine motorische Funktionsstörung auch durch eine Beeinflussung peripherer Regelmechanismen zustande kommt.

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24 January 1972.

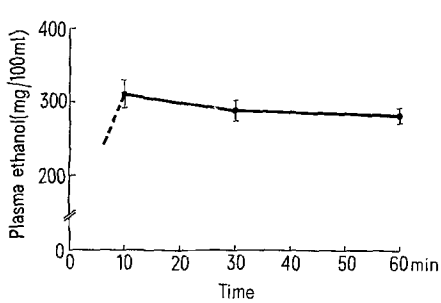

Fig. 3. Mean plasma ethanol levels in 15 anesthetized rats following ethanol (2.5 g/kg) administered i.p. Ordinate: concentration of ethanol in mg per 100 ml; ±1 standard error of mean is indicated by the brackets. Abscissa: time in min after the ethanol administration.

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