The Inhibitory Action of Caffeine on the Smooth Muscle of the Guinea-Pig *Taenia coli*

It has been reported that caffeine produces a contraction in skeletal muscle without membrane potential changes[1], and that caffeine acts on the sarcoplasmic reticulum and releases Ca from it into myoplasm[2]. I have reported that caffeine produced a contraction of smooth muscle at low temperature[3]. However, it is not likely, in smooth muscle, that caffeine acts on sarcoplasmic reticulum and causes a contraction as in skeletal muscle, because it is well known that the sarcoplasmic reticulum is very poorly developed in smooth muscle. Actually, it has been reported that caffeine caused an increase in smooth muscle tone followed by an increase in spike amplitude or spike frequency[3,4]. On the other hand, relaxation is observed if caffeine is applied under normal conditions or during potassium-induced contracture. The present experiments were performed to investigate this relaxing effect of caffeine.

Guinea-pig taeniae coli were used in the bathing solution of following composition: (m*M*) NaCl 133, KCl 5.6, $CaCl_2$ 2.5, $MgCl_2$ 1.1, $NaHCO_3$ 8.0, glucose 11.5, equilibrated with 95% O_2 and 5% CO_2. Contractions and relaxations of depolarized preparations were observed using kymographic techniques so that complete relaxations could be observed. Electrical activities and associated contractions were recorded by a double sucrose-gap method[5] and a mechano-electronic transducer (RCA 5734).

Contractures were induced by changing the bathing solution from normal to a potassium rich solution, in which all NaCl was substituted by KCl. Application of caffeine to the potassium-contracted preparation caused a remarkable relaxation. The threshold concentration to produce a relaxation was about 0.3 m*M* and complete relaxation, as compared to the relaxation induced by the deprivation of Ca by soaking in Ca-free solution containing 2 m*M* of ethylene glycol *bis*(-aminoethyl ether)-N, N-tetraacetic acid (GEDTA), was observed by the application of 5 m*M* of caffeine.

These relaxations were counteracted by increasing extracellular Ca concentration and relaxed muscles were reversed into contraction (Figure 1). In the presence of 5 m*M* of caffeine, the minimal concentration of additional Ca for the reversal was about 3 m*M* and complete reversals were observed by 25 m*M* of additional Ca. These results indicate the antagonizing action of caffeine to extracellular Ca in the contraction.

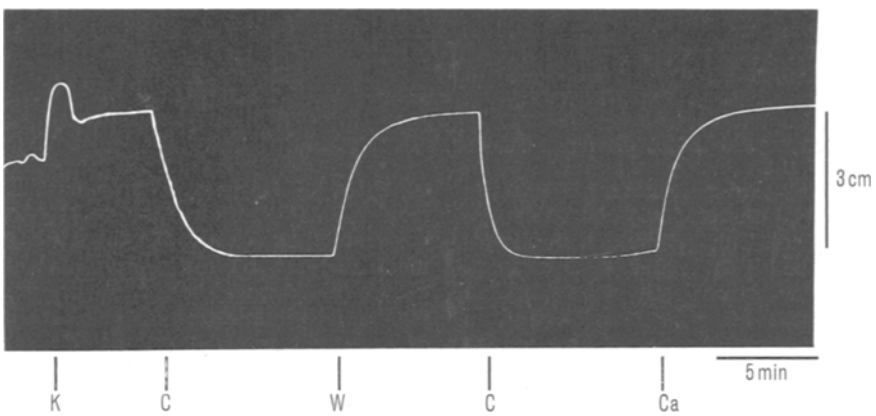

Fig. 1. The relaxing effect of caffeine and the reversal by Ca. Bathing solution was changed from normal to K-solution at *K*. *C*, *W*, and Ca indicate the application of 5 m*M* of caffeine, washing out of caffeine with K-solution, and addition of 25 m*M* of Ca in the presence of 5 m*M* of caffeine, respectively.

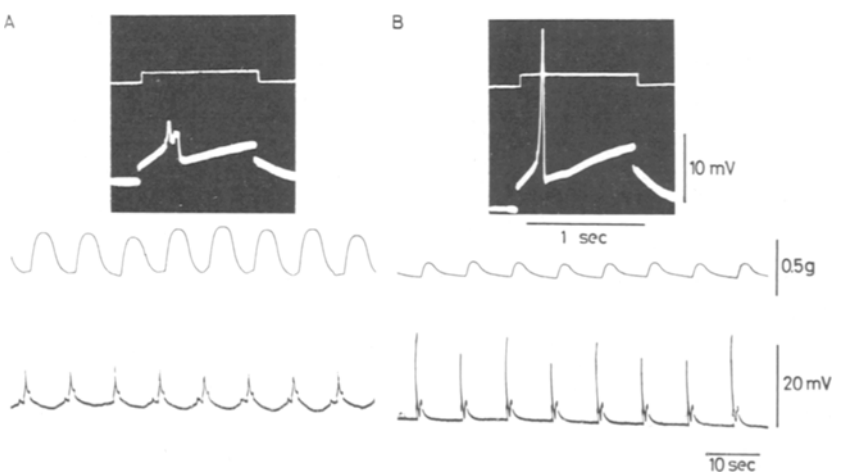

Fig. 2. The effects of caffeine on the electrical and mechanical activities. A, control. B, 20 min after the application of caffeine (5 m*M*). Action potentials and contractions were evoked by a depolarizing current of about 5×10^{-7} A (1000 msec duration) at a frequency of 1 per 10 sec. The upper photographs were taken at faster sweep speeds to show the shapes of the action potential.

The effects of caffeine on the electrical and mechanical activities of the taenia coli were also investigated. Initial increases in spike frequency and tension development were observed after application of caffeine but were not sustained: 5 min after the application of caffeine, gradual decreases in spike frequency and in tension were observed and, 10 min after, action potentials and associated contractions could be observed only by the application of depolarizing current. Conversely, spike amplitudes, which were observed during the application of depolarizing current, were increased. Irregular bursts of spike potentials observed before the application of caffeine became regular and well synchronized.

On the other hand, the tension developments which were initiated by the action potentials evoked by the depolarizing currents were decreased, regardless of the increase in the amplitude of the action potential. Thus, the partial dissociation of excitation and contraction was observed (Figure 2). These secondary effects of caffeine could be observed as long as caffeine was present, and were reversible.

These effects of caffeine were not affected by reserpinization[6] (i.p. injection of reserpine (10 mg/kg) 24 h and 12 h before performing the experiments), indicating that the inhibitory effects were not via the adrenergic inhibitory nerves.

Caffeine has dual action; first excitatory, and second inhibitory. The inhibitory action of caffeine can be explained from these results, by the assumption that caffeine affects the inward movement of Ca through the cell membrane, influencing the action potential and the contraction[7].

Zusammenfassung. Nachweis des muskelrelaxierenden (elektolischen und mechanischen) Effektes von Koffein auf den Meerschweinchendarm erst reizend und sekundär hemmend. Am K-kontrahierten Präparat führte Koffein zur Erschlaffung, die bei Erhöhung des extrazellulären Ca verändert wurde.

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Effect of Ingested Gossypol on the Growth Performance of Rats

The utilization of cottonseed as a source of dietary protein is greatly limited by the presence of a naturally-occurring pigment, gossypol. This compound, in its free state, has been shown to be highly toxic to non-ruminant species when ingested at levels existing in raw cottonseed meats, in solvent-extracted cottonseed meals, and in diets supplemented with these products. There is also some indication that the daily ingestion of feeds containing very low levels of gossypol, though not producing immediate overt symptoms of toxicity, may result in the accumulation of gossypol in various organs until a critical gossypol level is reached and toxic effects appear. The finding that gossypol binds with proteins and peptides, and particularly epsilon-aminolysine groups of these substances, has suggested that these gossypol complexes in the gut are not digested and absorbed, but are eliminated in the feces. Therefore, the quality of the protein in gossypol-containing diets is presumably reduced by the binding of the gossypol with the protein. Consequently, physiological disturbances observed in animals maintained on gossypol-containing diets having minimal protein requirements are probably not the result of gossypol toxicity, but the result of dietary protein insufficiency. Therefore, in order to investigate the effects of gossypol toxicity on physiological parameters, it is necessary to utilize diets which contain high quality protein in excess of the minimal protein requirements of the animal. This paper describes the growth performance of rats maintained on diets containing high concentrations of free gossypol and high levels of quality protein.

Materials and methods. The animals used in this study were 60-day-old, male rats of the CFE strain. Each rat was housed individually under uniform conditions of light (10 h light, 14 h dark) and temperature (68–72 °F). Animals were randomly divided into 3 groups corresponding to 3 diets: diets A, B, and C. The control diet (diet A) consisted of finely-ground commercial rat chow. The experimental diets were prepared by blending the ground commercial rat chow with solvent-extracted cottonseed meal obtained by the prepress solvent-extraction procedure (diet B), and with freshly-ground, raw cottonseed meats and vitamin-free casein (diet C). The composition of each diet was determined (Table I)[1–3]. Upon commencement of the feeding study, food and water were permitted ad libitum. Animals were weighed daily and their general appearance and condition noted. After 30 days, approximately half of each group was assigned to another study, and the remaining animals continued on the diets for a total feeding interval of 60 days.

Results and discussion. The results on the growth performance of rats receiving diets A, B, and C for feeding intervals of 30 and 60 days, respectively, are presented in Table II. The mean daily weight gains, final weights, and total weight gains of those animals maintained on diets B and C were significantly less ($P < 0.05$) than those maintained on diet A (control diet). These results substantiate the report that the ingestion of gossypol by non-ruminants results in a reduction of their growth rate[4]. It should be noted that the mean daily weight gain of those animals fed diet C for 30 and 60 days is not significantly different from the mean daily weight gain of those animals fed diet B, even

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