EFFECTS OF ANOMALOUS ANIONS ON THE CAFFEINE CONTRACTURE

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The mechanism linking the excitation (E) of the plasm membrane of striated muscle fibres with the contraction (C) of their myofibrils, i.e. E-C coupling (KAHN and SANDOW 1950), is a matter of great interest to many muscle physiologists. Recently, the following two interesting findings have been reported about the above problem: (a) the monovalent anion series, Br, NO₃ and I, increase the peak tension of the isometric twitch evoked by a single maximal shock without the change in the action potential (KAHN and SANDOW 1950, HILL and MACPHERSON 1954, RITCHIE 1954); (b) caffeine produces contracture by affecting the plasm membrane regardless of its electrical properties (AXELSSON and THESLEFF 1958). These evidences seem to suggest not only the important role of the plasm membrane in E-C coupling process, but also complicity of both the property and the function of the plasm membrane.

For clarification of E-C coupling process, the present authors studied the effect of the above anomalous anions (KAHN and SANDOW 1950) on caffeine contracture in detail, with particular consideration to the above two findings. The present paper is concerned with the detailed data on the brief survey of this subject that has been already reported (NAGAI 1959).

MATERIALS AND METHODS

The experiments were made at room temperature (about 18°C), using the isolated whole sartorius muscle of the frog (Rana japonica).

After the muscle was hung in the glass tube of about 10 ml in volume, which had a glass hook for fixing one end of the muscle. Caffeine dissolved in normal Ringer's fluid, K-Ringer's, anomalous anion Ringer's or anomalous anion K-Ringer's was applied to the muscle. The final concentration of caffeine in the above various Ringer's was 2×10⁻⁴w/v-1×10⁻²w/v. The evoked contractures were recorded on a slowly moving smoked drum with a light (0.5 gr) frontal lever.

The composition of the normal Ringer's was 116 mM NaCl, 2 mM KCl and 1.8 mM CaCl₂. In the K-Ringer's, all the Na was replaced by equivalent amount of K, and in the anomalous anion Ringer's and the anomalous anion K-Ringer's, all the Cl of NaCl or KCl was replaced by equivalent amount of monovalent anions (Br, NO₃, and SCN).

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RESULTS

In normal Ringer's fluid as well as in modified Ringer's, caffeine is capable of evoking contracture, and the contracture height under each condition is increased sigmoidally with the logarithmic rise in concentration of caffeine (Fig. 1). If an appropriate concentration of caffeine is used, the contracture is more marked in K-Ringer's than in the normal one (Fig. 1), and when anomalous anion is present, the contracture is more vigorous as compared with the case without anomalous anion (Fig. 1 and 2).

![Graph showing concentration-height-relationship of caffeine contracture under the conditions of various sorts of modified Ringer's fluids.](image)

**Fig. 1.** Concentration-height-relationship of caffeine contracture under the conditions of various sorts of modified Ringer's fluids.

\(\text{O}--\text{O}\), normal Ringer's; \(\bullet--\bullet\), NO₃ Ringer's; \(\triangle--\triangle\), K-Ringer's; \(\blacktriangle--\blacktriangle\), NO₃ K-Ringer's. Contracture heights in one sort of Ringer's are expressed by percentage of the height of the most vigorous contracture due to higher concentration of caffeine in the Ringer's. The heights in the above most vigorous case were the same regardless of the sort of Ringer's and were almost of the same order as tetanus height.

With these Ringer's fluids, the contracture produced by caffeine of lower concentrations soon relaxes spontaneously (Fig. 3) and, when caffeine is washed out in the course of contracture, it also relaxes reversibly for a few minutes (1-3 minutes) (Fig. 2). The contracture by intermediate concentrations of caffeine (higher than about \(2\times10^{-3}\text{w/v}\)) is, however, relatively complicated: the first contracture, which has a tendency of relaxation, is accompanied with the second contracture, which occurs in the course of relaxation of the first one. The second contracture has tendency not to relax but to sustain, and removal of the caffeine no longer causes relaxation (irreversible) (Fig. 3). If the concentration of caffeine is further increased, one strong contracture, which does
FIG. 2. Effect of anomalous anions on the caffeine contracture.

At 'a' and 'b', the same sartorius muscle was used. At marks (↑), various Ringer's fluids containing caffeine (1×10⁻³w/v) were given. Cl, Br, NO₃ and SCN indicate the sort of anions constituting the sodium salts (a) in Ringer's or the potassium salts (b). At the mark (↑) K-contracture was induced by replacing NaCl in Ringer's with equivalent amount of KCl. Relaxations (↓) were evoked by replacing with caffeine-free Ringer's, in which the anions to be tested at following addition of caffeine were contained, and their time courses, which continued for a few minutes, were not recorded as the result of stopping the rotation of the smoked drum.

FIG. 3. Caffeine contracture and the dependence of its time course on caffeine concentration.

a, 1×10⁻³w/v; b, 1.5×10⁻³w/v; c, 2×10⁻³w/v; d, 1×10⁻²w/v. Experiments were done in the normal Ringer's fluid. At R ↓ at 'c', caffeine was removed, but relaxation did not occur.

not relax, that is irreversible, is only recognized, as the result of the fusion of the second contracture to the first (FIG. 3).

In addition, the FIG. 1 illustrates the height of the first contracture as percentage of the maximum height of contracture produced by the high concen-
tration of caffeine. Considering the fact that, above a certain concentration of caffeine, the contracture consists of two phases, however, the above mentioned way of expression might be not always appropriate. In spite of such possibility, it is interesting that all the curves illustrated in the figure are of smooth sigmoidal shape.

**DISCUSSION**

By many investigations on the muscle model which consists of the actomyosin-system (Szent-Györgyi, 1951; Weber, 1958 and Nagai, 1956), it has well been established that a contractile system within muscle fibres is directly responsible for the mechanical process of muscular contraction. For understanding the mechanism in entire process of the muscular contraction, however, the studies on the process in E-C coupling, i.e. the mechanism linking the excitatory process of the plasm membrane with the contractile process inside the muscle fibres, are needed.

On this point, recent researches have given the following views: Kuffler (1946) and Katz (1950) have emphasized that the first of the linking process in question is the depolarization in the plasm membrane. On the basis of his findings that caffeine produces contracture of the skeletal muscle even at its concentrations from 0.08 to 0.1 per cent, which do not depolarize the plasm membrane, Taylor (1953) has supposed that the depolarization is not responsible for the E-C coupling process. Recent experiments made by Axelsson and Thesleff (1958) have shown that caffeine is capable of producing the contracture of the skeletal muscle even under the depolarized condition but cannot give rise to the contracture by its intracellular administration. Based on these results, they have concluded that the caffeine effect is due to the direct action of the drug on the E-C coupling process at the plasm membrane. Similar experiments to Axelsson and Thesleff using acetylcholine and other drugs have been made by Evans, Schild and Thesleff (1958) on the smooth muscle, and have led them to the similar view. Bay and Szent-Györgyi (1951) and Csapo (1954) have a different view from the above, emphasizing the direct role of action current in the E-C coupling process.

In the present results, it was recognized that the caffeine contracture was produced not only with normal Ringer's fluid but also with K-Ringer's, and this is quite similar to the results of Axelsson and Thesleff (1958). At least this fact, as is pointed out by them, suggests the presence of a process evoking the contracture without any electrical process at the plasm membrane.

As can be seen from Fig. 1, the curves to demonstrate the relationship of the height of contracture to logarithm of the concentration of caffeine are sigmoidal, and the rise in the concentration of the drug increases the contracture height. Though the meaning of the above relationship is not determined at
once, two possible explanations are considered: the one is based on the consideration that each muscle fibre has a proper sensitivity to caffeine, which varies quantitatively from fibre to fibre and responds to the drug in all-or-nothing law; the other is based on the consideration that the contractile behaviour of each fibre depends on the caffeine concentration. The parallel movement of the curves in Fig. 1 due to the conditions examined would be explained easily and reasonably on the basis of the first possibility; that is, the difference of the sensitivity of each fibre to the stimulant action of caffeine and the increasing effect of the anomalous anions on the sensitivity. On the other hand, based on the view of Kahn and Sandow (1955), that, of the two actions of the anomalous anions on the twitch, the threshold lowering action and the action on the contractile properties, the latter action is more responsible for the effect of the anions, the second possibility would be probable and this parallel movement of the curves would be explained by the sensitizing action of the anions on the contractile properties. Earlier, Gasser (1930) has pointed out that the contracture of each fibre due to drugs would not occur on the all-or-nothing law, and its intensity depends on the concentration of the drugs, which rather accords with the second possibility. Thus, the above anions effect might appear as the result of the potentiation effect of the anions on the contractile properties. For the clarification of the relationship between the caffeine contracture and the effect of the anions on it, however, further experiments should be made, using single muscle fibres.

As shown in Fig. 2 the caffeine contracture is enhanced by the anomalous anions with the following order SCN>NO₃>Br>Cl, which accords in the tendency with the result by Lillie (1910) that the contraction due to several drugs such as chloroform and saponin markedly was enhanced after treatment with several salts in the order of NaCl<NaBr<NaNO₃<NaI and NaSCN. These features are quite similar to the case of the twitch (Kahn and Sandow, 1950), suggesting the close relationship of caffeine contracture and some other contractures to the twitch with respect to their mechanism.*1 This deduction would support the Axelsson and Thesleff’s view (1958), which emphasized the resemblance between the caffeine contracture and the physiological contracture, on the basis of the reversibility*2 of the caffeine contracture and of the other fact shown by Hartree and Hill (1924) that the amount of heat released by caffeine in a muscle closely corresponded to the amount liberated by tetanic

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*1 Kahn and Sandow (1950) studied the effect of the anomalous anions with isometrical procedure. According to Hill and Macpherson (1954) and Kahn and Sandow (1955), a similar effect appears also isotonically. It is expected to be appropriate, therefore, to compare the present authors' results obtained isotonically with that by others isometrically.

*2 This reversible contracture is supposed to correspond to the first phase in the results of the present authors.
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stimulation.

According to KAHN and SANDOW (1950) and LUBIN (1957), anomalous anions enhance the peak tension of twitch by affecting the plasm membrane without appreciable change in the spike of the action potential. The present results in Fig. 1 and 2 show the anomalous anions enhance the caffeine contracture not only under the physiological condition (normal Ringer’s), but also under the condition of K-Ringer’s, indicating that the effect of anomalous anions has no connection with the electrical change at the plasm membrane; and thus the anomalous anions resemble caffeine with respect to the independence of their action on the electrical change at the plasm membrane. According to RITCHIE (1954), caffeine in relatively smaller concentrations and anomalous anions have a common action: they prolong the active state of the contractile element and thereby increase the twitch tension. SANDOW and KAHN (1952) has shown that K ion has a similar action to anomalous anions with respect to their accelerating effect on twitch, and Fig. 1 in the present paper demonstrates that such similarity is also recognized in the case of the caffeine contracture.

In view of the various points mentioned above, it is strongly suggestable that the above three agents, K ion, anomalous anions, and caffeine, have a common and closely related action on the skeletal muscle.

As mentioned above, it seems to have been received as a general view, that the prolongation of the active state due to the anomalous anions is responsible for the mechanism in the enhancement of the twitch due to the anions. The present results, however, show that the anions strengthen the caffeine contracture, the duration of which is so long that the intensity of the contracture would probably not depend upon the duration of the active state. Thus, it would be possible that the anomalous anions affect not only the duration of the active state but also the intensity of the state. MOMMAERTS et al. (1960) have recently emphasized that also the intensity of the active state should be taken into account for the problem about the grade of contraction.

It may be noted that the time course of the contracture due to caffeine in the range of intermediate concentration has two peaks regardless of the sort of Ringer’s fluid. The mechanism in the cause of the two peaks is at present being investigated by the present authors.

SUMMARY

The investigation of the effect of anomalous anions on the caffeine contracture was made, with the following results.

1. The caffeine contracture occurs not only in normal Ringer’s fluid but also in K-Ringer’s, and in the latter it is rather enhanced.
2. The caffeine contracture is enhanced by anomalous anions in the following order regardless of the sorts of Ringer’s, SCN>NO₃>Br>Cl.
3. The time course of the caffeine contracture is of double phase in the range of the intermediate concentrations of the drug, and this occurs regardless of the sorts of Ringer's and of whether the anomalous anions are present or not.

REFERENCES