The Influence of Caffeine on the Sodium Efflux in Barnacle Muscle Fibers

We[1,2] reported that injection of $CaCl_2$ into single barnacle muscle fibers stimulated the Na efflux in a K-containing or K-free solution. We also reported that lowering the external pH or injecting $CaCl_2$ stimulated the ouabain-insensitive component of the Na efflux. The provisional conclusion drawn from these observations was that the magnitude of the ouabain-insensitive Na efflux was governed by the internal concentration of free Ca^{2+}.

Because caffeine is known to raise the internal free Ca^{2+} concentration in skeletal muscle by increasing the influx of Ca^{2+} and by releasing the internally bound or sequestered Ca^{2+}[3,4], and because of the importance of establishing the sources of the increased Ca^{2+}, experiments with barnacle fibers were undertaken in the hope that caffeine would prove to be an effective probe.

The experiments were done using single fibers isolated from the depressor muscle bundles of the barnacle *Balanus nubilus* or *B. aquila*. The fibers were cannulated and then loaded with ^{22}Na by microinjection, using the technique devised by HODGKIN and KEYNES[5] as modified by CALDWELL and WALSTER[6]. The microinjector discharged a volume of ca. 0.1 µl of fluid per 1 cm excursion of the micromanipulator. The bathing medium used was artificial sea water the composition of which was the same as that employed previously[7]. Caffeine was obtained from Sigma Chemical Co.

The methods of measuring ^{22}Na in the effluent and the fiber were basically the same as described by BITTAR[8], and BITTAR, CALDWELL, and LOWE[9]. [^{22}Na]Cl was supplied by Amersham-Searle Corp. (SKS.-1). All experiments were carried out between 22 and 24°C.

In the first group of experiments, the effect of external application of 10 m*M* caffeine on the Na efflux was tested. In every case, the caffeine-treated fibers were found to promptly shorten to half their original length for periods of 2 to 3 min, followed by partial relaxation. Shown in Figure 1 is that the effect on the Na efflux produced by 10 m*M* caffeine was diphasic, the first phase being a prompt depression, followed by a marked rise in the Na efflux. This mode of behavior was noticed in 5 out of 7 fibers. In the remaining 2 fibers, an initial fall-off failed to occur; instead, there was a transient small rise in the Na efflux. Both of these results are in striking contrast to that reported by BITTAR, CALDWELL, and LOWE[9] who were unable to obtain with caffeine an effect on the Na efflux in crab muscle fibers. On average, the time required here for the onset of peak action by externally applied caffeine was 35 min. Estimates of the magnitude of this effect as calculated from the change in rate coefficient for ^{22}Na loss gave an average value of 131%, with a range of 59 to 132%. This seemed to be an underestimate, since calculations based on the change in slope of $d/dt\ ln\ d[Na^*]i/dt$ led to an average value of 180%.

In the second group of experiments, caffeine was applied internally in a concentration of 100 m*M*. Again, in every case there was some shortening of the fiber lasting about 2 min followed by complete relaxation. As shown in Figure 2, injection of a 1.5 cm column of the caffeine solution caused a prompt but small rise in the Na efflux. This rise, as calculated on the basis of the change in $1/Na^* \cdot dNa^*/dt$ averaged 26% ($n = 13$). The significance of this result with injected caffeine was not only that the rise in Na efflux was smaller but also that the initial phase of the diphasic effect observed with external application of caffeine was absent. The question thus posed was whether a raised internal Ca^{2+} resulting from increased Ca^{2+} influx caused by externally applied caffeine led to inhibition of the transport enzyme. To see whether the transient inhibitory action of caffeine depended on the presence of external Ca^{2+}, experiments were done using a Ca^{2+}-free solution. Again, each of the 6 fibers tested with 10 m*M* caffeine shortened to about half their initial length for 3 min, followed by partial relaxation. As il-

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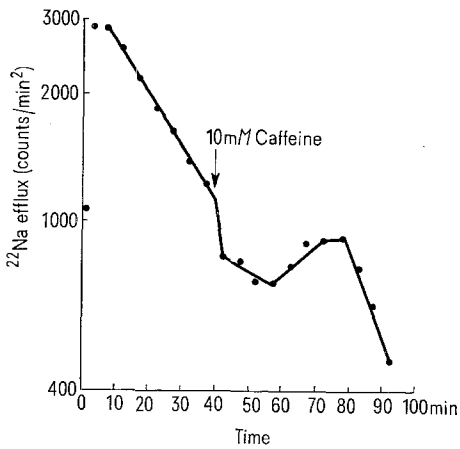

Fig. 1. The effects of external application of 10 m*M* caffeine on the Na efflux from a barnacle muscle fiber in artificail sea water, plotted semilogarithmically.

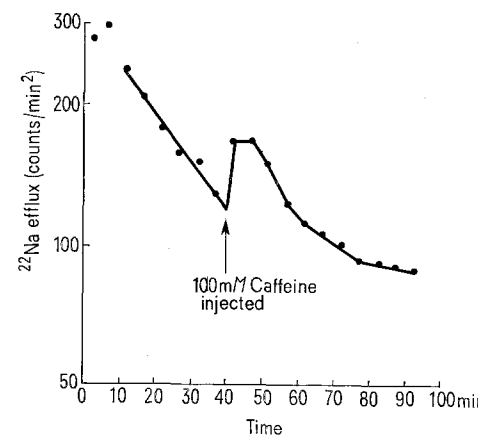

Fig. 2. The behavior of the Na efflux from a barnacle fiber in artificial sea water before and after the injection of 100 m*M* caffeine.

lustrated in Figure 3, introduction of 10 mM caffeine into the bathing solution caused a sudden decline, without any subsequent rise in the Na efflux. However, this was only true of 4 fibers, and in the remaining 2 the early phase consisted of a transient, small rise in the Na efflux. An additional 11 experiments showed that restoration of Ca^{2+} to the bathing medium brought about a rise in the Na efflux, the size of which averaged 125%. This result, which is illustrated in Figure 4, implies firstly that the threshold concentration for activation of the internal Ca^{2+}-sensitive component of the Na efflux is readily achieved in the presence of 10 mM external Ca^{2+} and secondly that the threshold internal concentration of Ca^{2+} for inhibiting the transport enzyme is achieved without external Ca^{2+} ions.

Knowing from previous experiments that the Na efflux in barnacle fibers is very sensitive to external acidification, the next logical step was to see whether the size of the effect caused by lowering the external pH is affected by prior external application of caffeine. A typical experiment given in Figure 5 shows that following an increase in Na efflux induced by caffeine, acidification brought about a further rise. The results based on 10 experiments indicated that 10 mM caffeine caused an average rise of 135% in the rate coefficient for Na efflux, as compared to a 367% rise caused by external acidification. Since normal fibers show a 450% rise in rate coefficient following a reduction in external pH from 7.8 to 5.8 (BITTAR, TONG, CHEN, and DANIELSON, unpublished data), it may be fairly judged that caffeine and protons (or CO_2) have to some extent a common action on the Na efflux.

The results of the present experiments with externally applied caffeine clearly show that stimulation by the alkaloid of the Na efflux depends on the presence of external Ca^{2+}. In addition, they show that stimulation is often preceded by inhibition, which cannot be avoided by simply removing Ca^{2+} from the bathing medium. The cause of the initial fall in the Na efflux may not be difficult to understand in the light of our working hypothesis that the sensitivities of the two components of the Na pump to an increased internal Ca^{2+} concentration differ in opposite ways. That is, one component, the Na^+-K^+ ATPase, is suppressed by a raised internal Ca^{2+} concentration, whereas the other, the ouabain-insensitive component, is stimulated by a raised internal Ca^{2+} concentration. Evidence favoring the idea of the existence of a Na efflux component which is stimulated by the injection of $CaCl_2$ has already been marshalled by us[10]. Why then does one obtain with caffeine inhibition and not stimulation of the Na efflux in the absence of external Ca^{2+}? Presumably because caffeine mobilizes sequestered or bound calcium, thereby leading to Ca^{2+} levels sufficient to inhibit the transport enzyme but not high enough to stimulate the second pump component.

It may now be asked, why is it that the injection of caffeine caused just a small rise in the Na efflux? The answer to this may lie in the idea that the Ca^{2+} level achieved by injecting caffeine is only enough to slightly

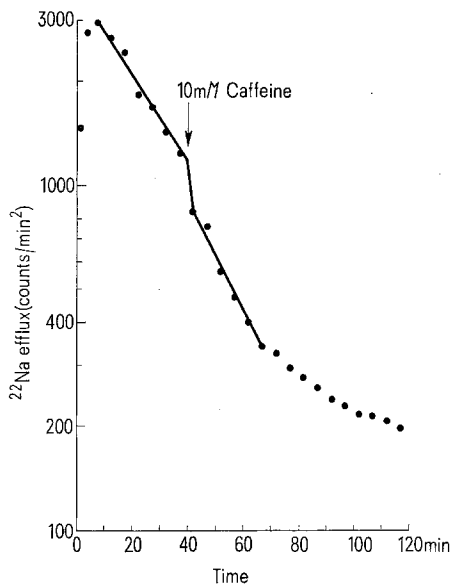

Fig. 3. The effect of external application of 10 mM caffeine on the Na efflux in a barnacle muscle fiber in Ca-free artificial sea water.

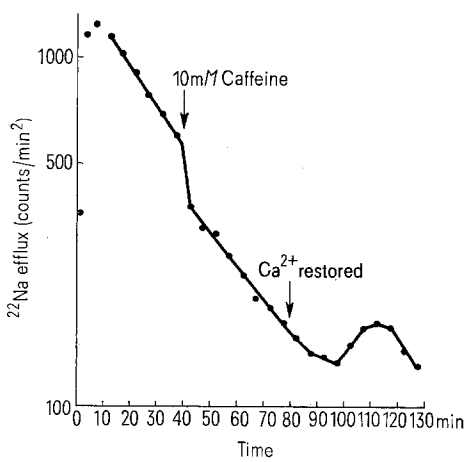

Fig. 4. The effects of external application of 10 mM caffeine on the Na efflux into a Ca-free solution and of restoring Ca^{2+} to the bathing medium 40 min after the addition of caffeine.

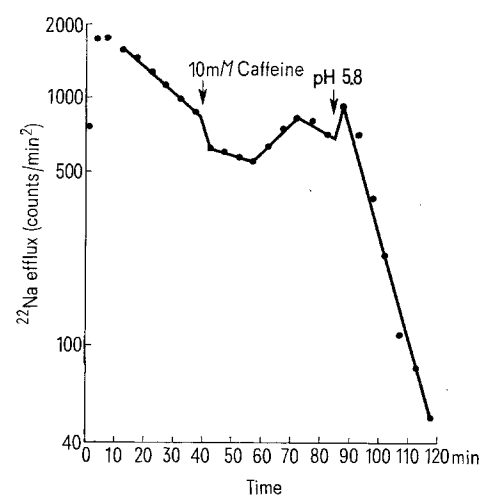

Fig. 5. The effects of external application of 10 mM caffeine on the Na efflux and of acidification 45 min after the addition of caffeine to the bathing medium.

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stimulate the second pump component. Other reasons, however, may be considered such as dilution of the caffeine by the myoplasm, and dilution of the specific activity of the internal radiosodium as the result of the release of sequestered Na[11].

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Zusammenfassung. Nachweis, dass Coffein zunächst den Auswärtstrom von Na-Ionen aus Einzelfasern der Bernakelmuskeln verringert, ihn hernach aber zu steigern vermag.

E. Y. TONG, E. E. BITTAR,
S. S. CHEN and B. G. DANIELSON

Department of Physiology,
University of Wisconsin,
Madison (Wisconsin 53706, USA),
17 January 1972.

Synaptic Frequency Demodulation

It is widely accepted that information is transmitted through nervous channels employing as a code the frequency of nerve impulses[1–3]. This code is demodulated at synapses into a quasi-analogue change of the post-synaptic membrane potential. This frequency-to-analogue conversion depends upon a great number of factors. Among these one can count pre-synaptic factors including presynaptic factilitation, potentiation and depression and also the statistical nature of the quantal release of transmitter. Post-synaptic factors include the duration of transmitter action, the membrane time constant, the membrane cable characteristics, and the non-linear summation of transmitter activity. Although a great deal is known about these various factors, it is not yet clear how all of them act in concert to produce synaptic frequency demodulation.

The neuromuscular junction is a well-known preparation where all the release parameters have been extensively investigated[4]. We here report preliminary results describing the dynamic characteristics of this synapse. We are currently engaged in defining the role of the various release parameters in determining synaptic frequency demodulation.

In the present study we employed the frog's (*Rana ridibunda*) sciatic nerve-sartorius preparation in vitro. Synaptic potentials were recorded intracellularly with conventional glass micropipettes, filled with 3 *M* KCl, which were connected through a cathode follower (Bioelectric Instruments) to the DC amplifier of a Tektronix 502A oscilloscope, and finally recorded on tape, using a Hewlett Packard FM tape recorder. The preparations were equilibrated in a medium containing a reduced calcium and increased magnesium ion concentration. In this medium the mean quantal content of the end plate potentials was low[5–7]. This served to prevent muscle contractions and also made the neuromuscular synapse resemble central synapses where synaptic potentials appear to have low quantal content[8,9]. For stimulation, a Wavetek waveform generator was connected to a frequency-modulation system which was employed to deliver supra-maximal stimuli to the sciatic nerve.

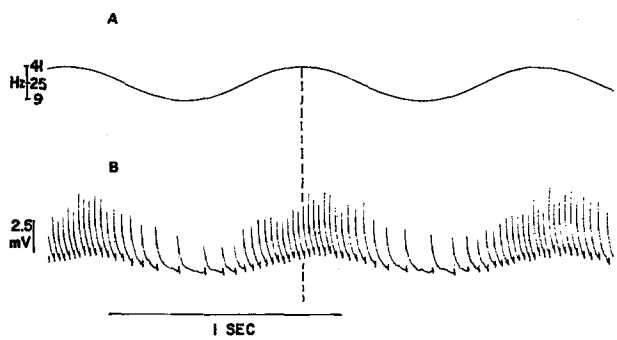

Fig. 1. Synaptic frequency to analogue conversion. The frequency of stimulation is shown as a continuous line, fluctuating between 41 and 9 isec. B) The post-synaptic membrane potential changes. Each impulse is a separate end-plate potential. Resting potential: 81 mV.

As can be seen in Figure 1, the frequency of stimulation of the sciatic nerve varied in a sinusoidal manner, each stimulus producing a distinct end-plate potential. In this particular experiment, the basic frequency of stimulation was 25 impulses/sec (isec) and was modulated in the range between 41 and 9 isec. The amplitude of the frequency modulation was thus 25 ± 16 isec (Figure 1A). It can be seen that both the base line of the post-synaptic potential and the peak amplitudes of the end-plate potentials varied in a sinusoidal manner, the membrane depolarization increasing with the rate of stimulation (Figure 1B). The pattern of frequency of stimulation of the nerve was thus translated into an analogue change in post-synaptic membrane potential. However, the post-synaptic changes show a small phase lag in respect to changes in the presynaptic rate of stimulation (see the dashed line denoting the peak frequency of stimulation).

It was next of interest to examine the amplitude response and the frequency response of the synapse. The results for amplitude response are shown in Figure 2. Here the amplitude of the frequency modulation was increased from 25 ± 0 isec (Figure 2A) through 25 ± 1.5 isec (Figure 2B), 25 ± 3 isec (Figure 2C), 25 ± 9 isec (Figure 2D) and 25 ± 16 isec (Figure 2E). It can be seen that,

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