LETTER TO THE EDITOR

A Reply to Godt and Baumgarten's Potential and K^+ Activity in Skinned Muscle Fibers: Evidence for a Simple Donnan Equilibrium Under Physiological Conditions

Dear Sir:

The K^+ ion-selective microelectrode measurements of Godt and Baumgarten (1984) have shown that K^+ is in electrochemical equilibrium at pH 7, which is close to physiological pH (pH 7.21 for crab muscle, Aickin and Thomas, 1975). In this observation they have confirmed the work of Collins and Edwards (1971) for rigor solutions and have extended this to relaxing solutions (in the presence of ATP). They have also confirmed the observation of Bartels and Elliott (1980, 1981, 1982) that the potential observed in the A-band with a 3 M KCl microelectrode is significantly more negative in the absence than in the presence of ATP. (We have noticed that random penetration of muscle fibers at moderate sarcomere lengths is much more likely to monitor the A-band than the I-band potential, so that under the conditions of Godt and Baumgarten's experiments the measured potential very probably arises from the A-band.)

Because we all agree empirically that there is electrochemical equilibrium at physiological pH for K^+, the major counter ion, in the presence of ATP or in its absence, then unless the major coion (Cl^-) is not in electrochemical equilibrium the analyses of Elliott and Bartels (1982) and Naylor (1982) show that it is legitimate to use the 3 M KCl potentials to calculate the fixed charge (i.e., the contractile protein charge) change between rigor and relaxed muscle. Nothing in Godt and Baumgarten's experimental observations vitiates this analysis or this conclusion.

Is it legitimate to assume that Cl^- is in electrochemical equilibrium at pH 7? In the absence of experimental evidence to the contrary, and given that K^+ is in equilibrium at this pH under all observed conditions, it seems highly unlikely that this assumption will be incorrect, but we shall await with interest any experimental reports using Cl^- ion-selective microelectrodes.

If the major counter and coions are in equilibrium and yet for any reason other diffusible ions are not so, will this cause any modifications in the fixed-charge measurements? Godt and Baumgarten give no argument to support such a hypothesis, beyond their sentences "the most likely explanation is that at pH 7 charge transfer associated with the ATPase reaction is fortuitously near balance, whereas at pH 5 it is not. This difference may arise from pH-induced alterations in the relative amounts and/or mobilities of the various diffusing species." But unless their K^+ electrode is sensitive to the diffusing species (and we doubt whether they themselves believe this), the K^+ balance surely shows that the amounts and/or mobilities of diffusing species are negligible at pH 7 compared with the electrochemical equilibrium. In this event, we know no physical principle that suggests that diffusion potentials are other than negligible, so that the situation reduces to an effective Donnan equilibrium, which leads to the fixed charge values that we have published already.

We are aware of the paper of Overbeek (1956) and the statement quoted from that paper. We do not, however, think that deviations from electrochemical equilibrium, which at pH 7 are too small to be measured with ion-selective electrodes, will cause

highly significant differences in the potentials observed with 3 M KCl microelectrodes between rigor and relaxation, which we have measured and which Godt and Baumgarten confirm. We shall continue to regard these potentials as Donnan potentials and the calculated fixed-charge differences at physiological pH as real unless Godt and Baumgarten demonstrate theoretically or empirically that this is not the case.

Godt and Baumgarten's results show that under the ionic conditions of their solutions the effective isoelectric point is ~pH 5.8, both with and without ATP. This agrees with similar results for frog muscle by Dewey et al. (1982). In addition, Godt and Baumgarten have made some most interesting observations at low pH. Close to the isoelectric point, but on the alkali side (at pH 6) their results show that K^+ is in equilibrium in the absence of ATP, and very nearly so in its presence. Their results on the acid side of the isoelectric point (at pH 5) again show a K^+ equilibrium in the absence of ATP, but a significant disequilibrium in the presence of ATP. However, we think they should consider some possible perturbation factors before they relate their observation to working muscle. In the first place, working muscle includes a buffering power of 47 slykes (mM H^+ added/pH change produced) that resists change to lower pH (Aickin and Thomas, 1975). The buffering power of 15 mM Tris maleate at pH 5, measured in solutions made up according to the data given in Godt and Baumgarten (1984), is only ~2.5 slykes in the acid direction (this pH is outside the recommended range for the buffer), so that the buffering is unlikely to be very effective at pH 5 under these circumstances (see Murphy and Koss, 1968). It therefore seems possible that H^+ ions produced by ATP splitting might induce appreciable fixed-charge changes and thus cause local K^+ disequilibrium. In the second place, it seems possible that organic compounds, released at low pH from mechanically-skinned fibers in the presence of ATP, might interfere with the K^+ electrode. In this connection Dawson and Djamgoz (1983) have shown that K^+ ion-selective electrodes using the Corning ion-exchanger 477317 resin give abnormal readings in blow fly larval muscle cells (compared with electrodes using the valinomycin-based neutral ion carrier [NIC]). They report that in these muscles the 477317 electrode potentials lead to an internal potassium activity 26% greater than that derived from the NIC electrode potentials (this is a very similar excess to that shown in Godt and Baumgarten's Table III at low pH in the presence of ATP), and they conclude that the 477317 electrodes are sensitive to an organic interfering ion.

Even if both these effects turn out to be negligible, however, we shall still doubt whether phenomena that have been observed close to the isoelectric point or on the acid side of that point are important for the behavior of working muscle at physiological pH. We would prefer to look for an explanation in the work of Sarkar (1950), who measured the effects of ATP and ions on the isoelectric point of myosin, and showed that this point was highly dependent on the added ions, presumably because of specific ion

absorption onto the protein. Dewey et al. (1982) have observed shifts of isoelectric point in some muscles, and point out that different buffers also produce different detailed effects, and they mention ion absorption as a probable explanation. The effects that Godt and Baumgarten observe may well be explicable in these terms.

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