Quinine Stimulation of Ca^{45} Efflux from Arthropod Skeletal Muscle in Relation to Quinine Effects on Fibre Calcium Translocation and Binding[1]

It is now generally accepted that contraction and relaxation in vertebrate skeletal muscles are activated by a cyclical rise and fall in the free calcium content of the fibres, this being accompanied by a corresponding rise and fall in fibre calcium efflux[2,3]. Several alkaloid drugs which enhance contraction in vertebrate skeletal muscle have been shown to stimulate fibre calcium efflux[4–7], and this has also been shown for caffeine action on barnacle giant muscle fibres[8]. Studies so far have not attempted to relate observed alterations of calium efflux to changes in intracellular calcium binding drug-induced contracture activity. Quinine has been shown to induce contractures in arthropod skeletal muscle[9], and in this present study we have attempted to relate quinine action on calcium efflux and quinine action on fibre calcium binding to the observed contracture-induction effects of this alkaloid.

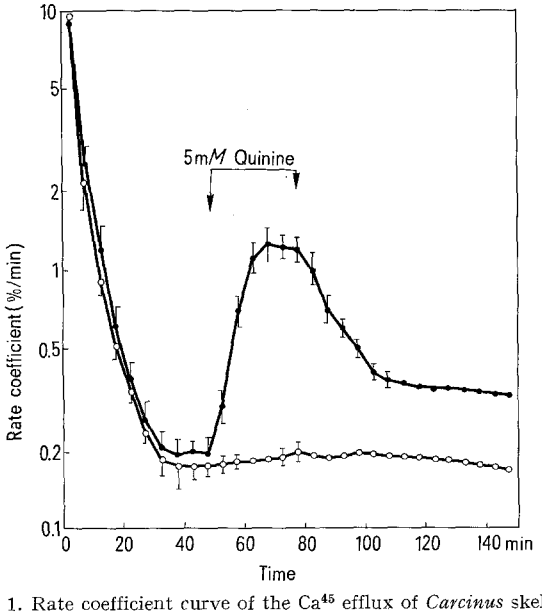

Fig. 1. Rate coefficient curve of the Ca^{45} efflux of *Carcinus* skeletal muscle fibres. Open circles represent control efflux conditions and closed circles show the effect of 5 m*M* quinine on the efflux. Note that quinine causes a massive stimulation of the slow efflux phase of intracellular origin, indicating a large rise in fibre free calcium. Each point is the mean ± S.E. (n = 3 for controls, n = 6 for quinine treatments).

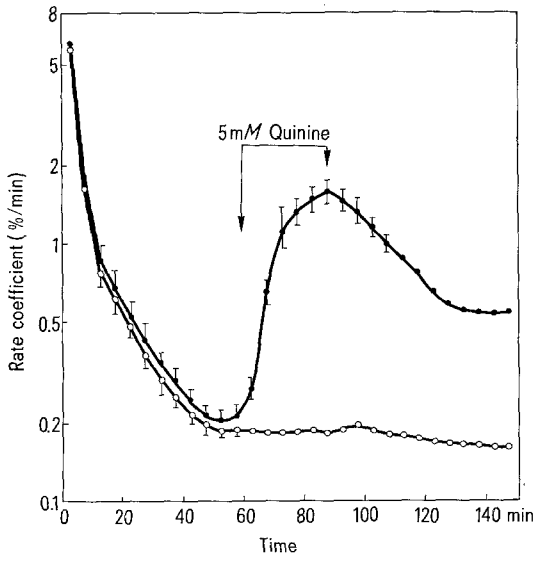

Fig. 2. Ca^{45} efflux curves of *Periplaneta* skeletal muscle fibres under the same conditions as Figure 1. Again note the massive quinine-induced stimulation of the slow intracellular phase of Ca^{45} efflux. Each point is the mean ± S.E. (n = 3 for controls, n = 6 for quinine treatments).

Materials and methods. For efflux experiments, small bundles of muscle fibres were dissected from the coxae of the cockroach (*Periplaneta americana*) and from the claw closer muscle of the common shore crab (*Carcinus maenas*). The fibres were loaded for 1 h in standard salines, consisting of NaCl 520, KCl 10, $CaCl_2$ 12, $MgCl_2$ 8, $NaHCO_3$ 3, sucrose 100 for *Carcinus*, and NaCl 140, KCl 10, $MgCl_2$ 2, $CaCl_2$ 2, NaH_2PO_4 6, $NaHCO_3$ 4, sucrose for *Periplaneta* (all in m*M*). These salines contained 4 μCi/ml Ca^{45}. After loading, the preparations were placed in a constant flow apparatus, the saline leaving the preparations being collected at 5 min intervals on a conventional fraction collector. The quinine (5 m*M*) was added to the experimental salines as indicated in the results sections. The Ca^{45} activity in each fraction and that remaining in the fibres after ashing at 500 °C was estimated in a Packard liquid scintillation counter, and from these data the rate coefficient of the efflux was calculated[6]. Further details of these methods will be published elsewhere[10].

To determine cellular calcium binding, muscles were homogenized in 100 m*M* KCl and 1 m*M* imidazole, and the mitochondria and sarcoplasmic reticulum (SR) were isolated and purified by differential centrifugation. These are the muscle cell compartments known to be responsible for fibre calcium regulation in the contraction-relaxation cycle[11,12]. Isolated SR and mitochondria were allowed to bind Ca^{45} in calcium incubation media consisting of 4 m*M* $MgCl_2$, 2 m*M* ATP (disodium salt) in 40 m*M* *tris* maleate buffer at pH 7 (NAKAMARU and SCHWARTZ[13]). The calcium content of the incubation media was varied as shown in the results section, and the Ca^{45} activity of the incubation media was adjusted to about 50,000 cpm. After incubation in control and quinine-containing (5 m*M*) media, SR and mitochondria were separated from the loading solution on membrane filters (0.45 μm pore), washed, and their Ca^{45} activity was determined by liquid scintillation counting. Details of these techniques have already been published[14].

Results. The control Ca^{45} efflux curves for *Carcinus* and *Periplaneta* skeletal muscle fibres are shown in Figures 1 and 2 respectively. It can be seen that these curves

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resolve into 2 components, an initial fast component and a later slow component, corresponding to extracellular readily diffusible calcium and calcium of intracellular origin respectively[5]. When the perfusion saline was changed for one containing 5 m*M* quinine during the efflux of intracellular origin, a massive stimulation of Ca^{45} efflux was seen. In the case of *Carcinus*, the stimulation was from a rate coefficient of 0.2%/min to 1.3%/min, figures for *Periplaneta* being from 0.2 to 1.8%/min. This massive stimulation of efflux lasted for the duration of drug application and after quinine removal the efflux settled down to a level significantly higher than that seen in the controls (0.4%/min compared with 0.2%/min in *Carcinus* and 0.55%/min compared with 0.18%/min in *Periplaneta*). These results show that quinine has had a considerable stimulation effect on the level of muscle fibres free calcium in both preparations, and the question arises as to what calcium compartments within the cell have been affected by the drug.

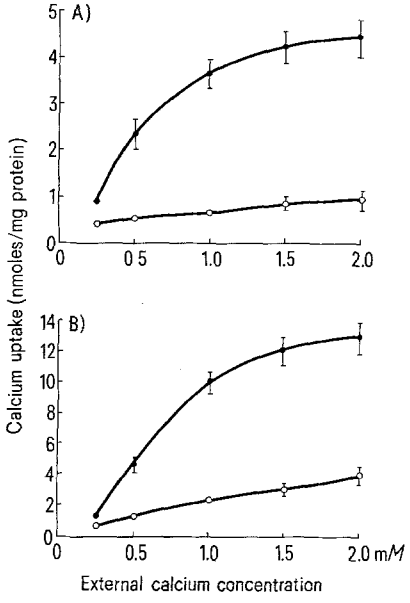

Fig. 3. Calcium uptake by mitochondria (A) and sarcoplasmic reticulum (B) isolated from *Carcinus* skeletal muscle. Filled circles, control conditions; open circles, uptake in 5 m*M* quinine media. Note the massive inhibition of calcium binding caused by quinine. Each point is the mean ± S.E. ($n = 10$).

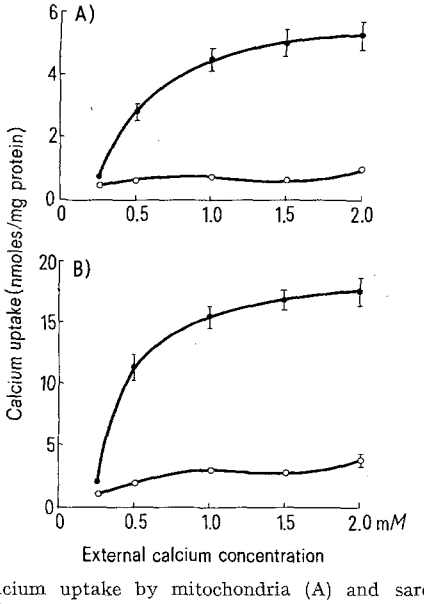

Fig. 4. Calcium uptake by mitochondria (A) and sarcoplasmic reticulum (B) isolated from *Periplaneta* skeletal muscle. Filled circles, control conditions; open circles, uptake in 5 m*M* quinine media. Again note the massive inhibition of calcium binding caused by quinine. Each point is the mean ± S.E. ($n = 10$).

The most likely muscle fibre organelles to be affected by quinine are those which are known to actively bind calcium, namely the sarcoplasmic reticulum and the mitochondria. To test this hypothesis, suspensions of sarcoplasmic reticulum and mitochondria were isolated from *Carcinus* and *Periplaneta* muscles, and their calcium binding properties were examined under control conditions and under the influence of quinine. The results of these experiments are summarized in Figures 3 and 4. Of the 2 muscle fractions examined, the sarcoplasmic reticulum had by far the highest calcium binding capacity in both *Carcinus* and *Periplaneta* (Figures 3B and 4B), but the mitochondria still possessed some considerable powers of calcium uptake (Figures 3B and 4A). It was found that incubation of reticulum and mitochondria from both *Carcinus* and *Periplaneta* in media containing Ca^{45} plus 5 m*M* quinine caused a considerable inhibition of calcium binding by both compartments, this inhibition reaching levels of 90% in most preparations examined.

Discussion. A large rise in calcium efflux is known to be associated with contractile activity in skeletal muscle fibres[2,3], and the massive increase in Ca^{45} efflux seen in these quinine treated fibres is clearly related to quinine's ability to induce contractures in arthropod skeletal muscle[9]. This massive increase in Ca^{45} efflux clearly mirrors a steep rise in myoplasmic free calcium, a known prerequisite for contraction. The results of the second part of this study show that quinine-induced inhibition of reticular and mitochondrial calcium binding is severe enough to adequately account for the observed rise in myoplasmic free calcium and increase in Ca^{45} efflux. The way in which quinine brings about reduction in reticular and mitochondrial calcium binding and thus a rise in myoplasmic free calcium is not clearly understood. This inhibition could be by a direct competitive effect at the membrane ATPase binding sites (see VAN DER KLOOT[15]), but the irreversible nature of quinine's contractile actions (HUDDART[9]) seems to argue against this hypothesis. However, as yet little is known about the binding of quinine or other alkaloids to subcellular membranes. Alternatively, quinine may inhibit calcium uptake by modifying the nature of the calcium binding proteins of the reticulum and mitochondria (the ATPase and cal-sequestrin protein fractions[16]). In a recent study of isolated reticular and mitochondrial proteins, separated by polyacrylamide gel electrophoresis after SDS (sodium dodecyl sulphate) dispersal[12], quinine was found to cause a virtual elimination of the 95,500 molecular weight ATPase band and the acidic protein bands in mitochondria, and to cause a massive reduction of these bands in sarcoplasmic reticulum. These findings suggest a direct non-competitive disruptive action by quinine on the loci of calcium binding in reticular and mitochondrial membranes. However, further work on quinine binding to cellular fractions and on the physical effects of quinine on isolated individual reticular and mitochondrial proteins will be required to confirm this hypothesis.

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Zusammenfassung. Chinin (5 m*M*) ruft einen starken Anstieg des Ca^{45} Ausflusses aus den Skelettmuskelfasern von *Carcinus* und *Periplaneta* hervor. Bei dieser Konzentration bewirkt Chinin eine ausgeprägte Hemmung der Kalziumbindung am isolierten sarcoplasmatischen Reticulum und der Mitochondrien dieser Muskeln. Die Hemmung zellulärer Kalziumbindung steht in Wechselbeziehung mit dem Anstieg freien Kalziums und der durch Chinin bewirkten Kontraktionsaktivierung.

H. Huddart and Mary West

Department of Biological Sciences, University of Lancaster, Lancaster, LA1 4YQ (England), 22 January 1975.

^{3}H-Nortriptyline Uptake and Tissue-Binding in vitro and its Effect on ^{3}H-Noradrenaline Uptake

Tricyclic antidepressants of the nortriptyline (NT) type are known to inhibit the uptake of noradrenaline (NA) into NA nerve terminals both in the peripheral and central nervous system[1–4]. This effect of NT is probably of decisive importance for the antidepressive effect of the drug[5]. NT is a highly lipid-soluble compound and is readily taken up into various tissues and easily passes the blood-brain barrier. With this in mind, it was of interest to study whether NT bound to brain tissue is of importance for the uptake of NA into NA nerve terminals. The present experiments were designed to shed some further light on drug binding to tissue components and to plasma proteins[6]. This binding of drugs is of special interest in in vitro experiments. Clinically it is also important to know if a tissue-bound drug has an inhibitory effect on the uptake of NA.

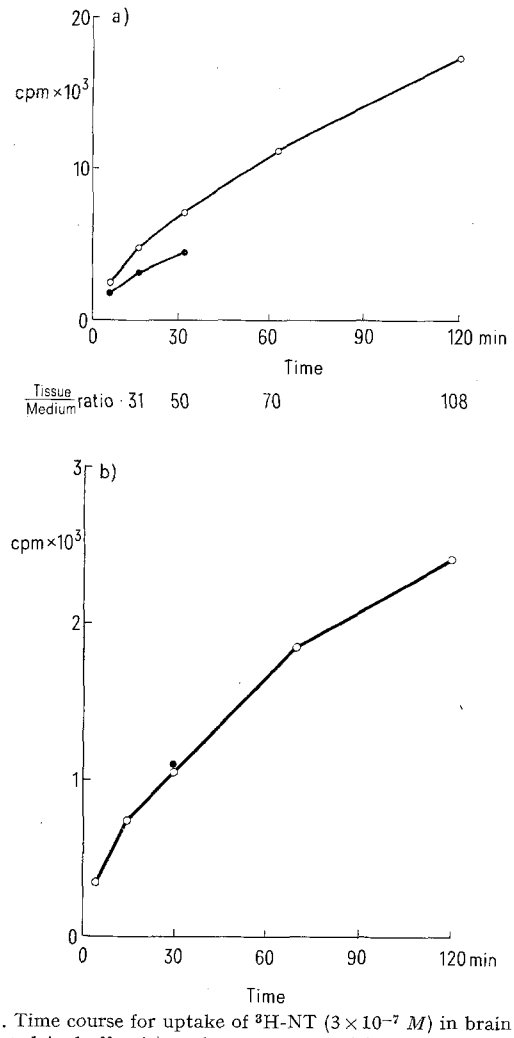

Fig. 1. Time course for uptake of ^{3}H-NT (3×10^{-7} *M*) in brain slices incubated in buffer (a) or human plasma (b). Open circles, 1 mg tissue per ml medium. Closed circles, 10 mg tissue per ml medium. The tissue/medium ratio for uptake in buffer, 1 mg tissue/ml is shown in a). Each value is the mean of 4 determinations.

Methods. Coronal brain slices (0.5 mm thick, 3 mm diameter, weight about 5 mg) from untreated female Spraque-Dawley rats were used. The slices were incubated either in a Krebs-Ringer bicarbonate buffer or in human plasma obtained from the bloodbank. ^{3}H-NA (HCl, 5–10 Ci/m*M*), ^{3}H-NT (HCl, 165 mCi/m*M*), kindly donated by Dr. O. Borgå, and unlabelled nortriptyline (Pharmacia, Sweden) were used in the experiments[4]. The radiochemical purity of ^{3}H-NT was checked with SiO_2 thin layer chromatography (2 *N* ammonia: methanol, 1:4) and was found to be more than 90%.

After the various incubation procedures, the tissue was rapidly rinsed in buffer and the radioactivity determined after solubilization with Soluene® and addition of toluene scintillation solution. Efficiency was determined after addition of ^{3}H-toluene.

Results and discussion. ^{3}H-NT accumulates rapidly in brain slices during incubation in buffer (Figure 1a). After 2 h, a more than 100-fold accumulation of radioactivity has appeared in the slices as compared to the medium. It is reasonable to believe that accumulation of ^{3}H-NT in contrast to ^{3}H-NA accumulation[3] is non-specific and not energy-dependent. The rapid accumulation of NT is most likely related to its high lipid-solubility. When the amount of tissue is increased in the incubation medium from 1 mg to 10 mg tissue/ml medium, the accumulation of ^{3}H-NT in the tissue is less prominent, due to a decrease of the concentration of ^{3}H-NT in the medium (Figure 2). When the incubation is performed in human plasma, ^{3}H-NT accumulation in tissue is almost 10 times less effective (Figure 1b), because more than 90% of ^{3}H-NT is bound to plasma proteins[6]. This implies that both the 'free' concentration of NT in plasma and the accumulation of ^{3}H-NT in slices incubated in plasma are reduced to about 10% compared to the buffer experiments. There seemed to be a fairly rapid equilibration between the 'free' and the plasma protein bound fractions of ^{3}H-NT, since 1 and 10 mg tissue/ml plasma results in the same ^{3}H-NT accumulation in the slices. Thus NT bound to plasma proteins is a reserve pool for the drug.

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