We have previously reported that replacement of chloride with isethionate also evokes a large increase in labelled GABA from the superfused dorsal medulla[9]. This is probably also due to neuronal release, since in a chloride-free extracellular medium neurones would be likely to lose intracellular chloride, thus depolarizing and increasing release of neurotransmitter.

The criterion of neuronal release is one of particular importance in the identification of a neurotransmitter. Since in vitro slices and homogenate fractions may not necessarily behave as does intact tissue, it is important to study the in vivo as well as the in vitro preparation. The data presented here provide good evidence that in the intact dorsal medulla, labelled GABA can be released from a neuronal pool. We consider it unlikely that stimuli as diverse in their mode of action as veratridine, extracellular chloride replacement, electrical depolarization and high potassium could have in common similar effects on other but neuronal tissues, and conclude that the release of GABA demonstrated here is from a neuronal source.

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2 N. Davidson and C.A.P. Southwick, J. Physiol. *219*, 689 (1971).
3 N. Davidson and H. Reisine, Nature New Biol. *234*, 233 (1971).
4 N. Davidson, E.I. Macfarlane and D.L. Michie, Experientia *33*, 935 (1977).
5 P.J. Roberts, Brain Res. *67*, 419 (1974).
6 R.G. Hill, J.F. Mitchell and F. Roberts, Br. J. Pharmac. *60*, 274P (1977).
7 J.A. Assumpção, N. Bernardi, C.G. Dacke and N. Davidson, Br. J. Pharmac. *59*, 488P (1977).
8 N.G. Bowery and M.J. Neal, J. Physiol. *275*, 58P (1978).
9 N. Bernardi, J.A. Assumpção, N. Davidson and C.G. Dacke, Experientia *33*, 913 (1977).

The origin of the instantaneous elasticity in single frog muscle fibres

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Summary. High-speed cinematographic recordings of single tetanized muscle fibres during a quick decrease in length revealed that the shortening was mostly localized at the fibre segment nearest the released end of the fibre, indicating that the instantaneous elasticity may not originate from the elasticity of the cross-bridges.

In 1971, Huxley and Simmons[2,3] studied the tension changes in single frog muscle fibres following quick length changes which were complete within 1 msec, and presented evidence that the instantaneous elasticity as determined by the above method may largely reside in the cross-bridges. According to them, each cross-bridge consists of a myosin head and an elastic link extending from the thick filament, and the straight length-tension relation of the cross-bridge elasticity is truncated by the early tension recovery due to the rotation of the myosin head[3-5]. As a matter of fact, the length-tension relation of the instantaneous elasticity is straighter, the larger the velocity of quick decrease in length[2,5]. In order to ascertain whether the instantaneous elasticity actually originates from the cross-bridges, the most straightforward way may be to record the length changes in different parts of the fibre during the course of a quick change in length. The present experiments were undertaken to examine the origin of the instantaneous elasticity by use of ultra high-speed cinematography.

Material and methods. Single muscle fibres (diameter, 50–120 µm) were isolated from the semitendinosus muscles of the frog *(Rana japonica)*, and a pair of stainless-steel wire connectors (0.1 mm in diameter and 2–3 mm in length)[6,7] were tied to both tendons with braided silk thread. Then, the fibre was mounted horizontally by hooking the connectors to the force transducer (Aksjeselskapet Mikroelektronikk, AE80, resonance frequency, 3 kHz) and the lever of the displacement transducer (light beam-photodiode system)[6,7] (figure 1A, inset). The fibre was held at the slack length L_0 (0.8–1.2 cm, excluding tendons), and tetanized maximally by applying transverse alternating currents (50 Hz) through a pair of Pt plates at both sides of the fibre. When the maximum isometric tension P_0 (2.5–3 kg/cm^2) was developed, the fibre length was changed quickly by pushing the lever of the displacement transducer with a vibrator (Ling, type 203), the velocity of length change (1% change was complete in 0.12–0.20 msec) being equal to or a little larger than that used by Ford, Huxley and Simmons[5]. The length and tension changes were monitored with a dual-beam oscilloscope. A number of fine carbon particles were firmly attached to the fibre surface, and the length changes of the fibre segments divided by the particles were recorded during the course of quick length changes with a 35-mm ultra high-speed cinecamera (Beckman, Model 165) at 40,000–50,000 frames/sec (figure 1B)[8]. Attention was focused on the length changes of the fibre segments until the total fibre length shortened by 0.5–1%, since the tension is known to drop from P_0 to zero within the above range of quick decreases in length[3-5]. This was also confirmed in the present study, though the frequency response of the force transducer was not high enough to follow the exact time course of the tension changes. All experiments were made at room temperature (18–22 °C).

Results. At first, it was found that, if the connector was simply hooked to the lever imposing length changes on the fibre, the fibre did not shorten quickly while the lever was moving quickly as shown in figure 1A; since the diameter of the hole (0.8 mm), through which the connector was hooked to the lever, was many times larger than the diameter of connector wire, the lever could move in the direction of fibre shortening for a distance while the position of the connector did not change appreciably (figure 1A, inset). This indicates that the instantaneous elasticity can recoil only with a velocity much slower than that of the lever movement used, when the fibre is suddenly allowed to shorten freely.

To prevent the above detachment of the connector from the lever, the connector was firmly clamped to the lever, and similar experiments were repeated. Figure 1C shows a typical example of the length changes of the fibre segments

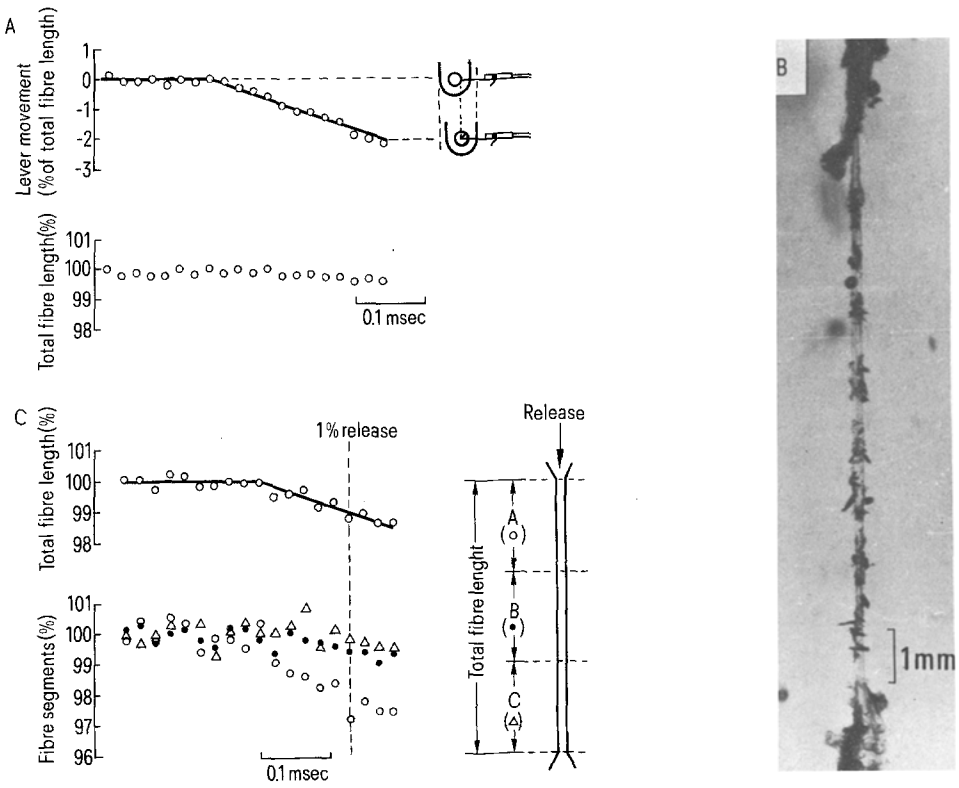

Fig. 1. Ultra high-speed cinematography of single frog muscle fibres during the course of quick decreases in length. *A* A marked difference in the time course of the lever motion and the shortening of the fibre, indicating the detachment of the connector from the lever when the former was simply hooked to the latter as illustrated in the inset. *B* A frame from a cinefilm of a tetanized fibre during a quick change in length. A number of carbon particles are attached to the fibre surface. *C* Time course of length-changes of the fibre segments during a quick decrease in length when the connector was clamped to the lever. The fibre was divided into 3 segments of nearly equal lengths (A, B and C in the inset). The total length change of the fibre is also shown. Note that the shortening is mostly localized in the segment A nearest the released fibre end.

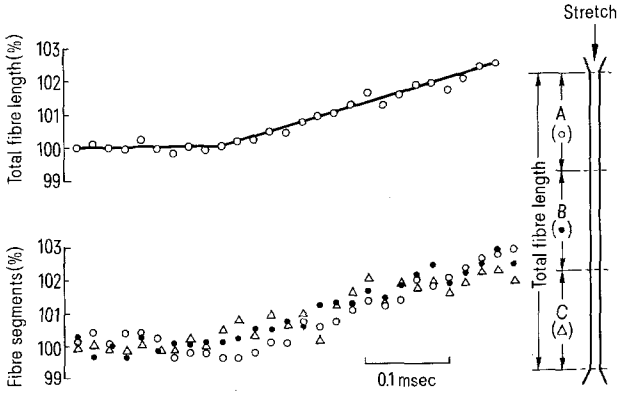

Fig. 2. Length changes of the fibre segments during the course of a quick increase in length. The fibre was also divided into 3 segments of nearly equal lengths (A, B and C in the inset). Note a fairly uniform lengthening along the fibre length.

during a quick decrease in length. It can be seen that, until the total fibre length shortens by 0.5–1%, the shortening is not uniformly distributed along the entire fibre length, but is mostly confined to the fibre segment nearest the released end of the fibre. This seems to indicate that the drop of tension during a quick decrease in length is not due to the elastic recoil of the cross-bridges, but is due to the localized buckling of the fibre at the released end. Since the transmission velocity of mechanical impulse along the fully activated muscle fibres is reported to be 17 cm/msec[9], the mechanical transmission time should be definitely shorter than the time within which the fibre shortened by 1%. When the fibre was stretched quickly, on the other hand, the lengthening of the fibre segments was observed to take place fairly uniformly along the fibre length as shown in figure 2. Though it was sometimes noticed that the rate of lengthening was somewhat greater at the segment nearest the stretched fibre end than at the other segments, the difference was much smaller than that observed during quick decreases in length. It still remains to be investigated whether the quick stretch mainly produces the elastic extension of the cross-bridges or produces the extension of myofilaments as well.

In conclusion, the tension changes resulting from too rapid length changes may not give correct information about the cross-bridge properties, and the nature of the instantaneous elasticity should be reexamined from this point of view.

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2 A.F. Huxley and R.M. Simmons, Nature *233*, 533 (1971).
3 A.F. Huxley and R.M. Simmons, J. Physiol. *218*, 59P (1971).
4 A.F. Huxley and R.M. Simmons, Cold Spring Harb. Symp. quant. Biol. *37*, 669 (1972).
5 L.E. Ford, A.F. Huxley and R.M. Simmons, J. Physiol. *269*, 441 (1977).
6 M.M. Civan and R.J. Podolsky, J. Physiol. *184*, 511 (1966).
7 H. Sugi, J. Physiol. *225*, 237 (1972).
8 T. Tameyasu and H. Sugi, Experientia, in press (1978).
9 M. Shoenberg, J.B. Wells and R.J. Podolsky, J. gen. Physiol. *64*, 623 (1974).