plastic behaviour; its viscosity decreases as the shear rate increases (anomalous viscosity). Furthermore, the non-Newtonian behaviour may explain the different values of viscosity obtained with measurements using different flow patterns. Therefore it is important to determine exactly the flow patterns and the shear rates when values of plasma viscosity measurements are compared and interpreted.

The results obtained with the Wells-Brookfield cone-plate viscometer show the poor accuracy of this type of viscometer at low viscosity levels[7,12]. Although care has been taken in our investigations to minimize the influence of surface tension, which might affect the readings in Low Shear 30[4,8], there is a considerable standard deviation at the lowest shear rates (0.017 and 0.059 sec^{-1}) which might be due to the particular viscoelastic properties of the macromolecules in plasma. In order to characterize the rheological properties of plasma, it is necessary to perform measurements of its viscosity at very low shear rates, where the viscosity is anomalous. Sequential measurements of red blood cell velocity in nailfold capillaries of man[13] demonstrated that very low velocities do occur intermittently in the healthy subject. Since plasma viscosity is a prime determinant of the flow in small blood vessels and in capillaries, its measurement at different shear rates will be important for the understanding of blood flow regulation in microcirculation.

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Evidence for a neuronal release of isotopically labelled γ-amino-n-butyric acid (GABA) from the rat dorsal medulla in vivo[1]

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Summary. High potassium and electrical stimulation consistently increase efflux of labelled GABA from the in vivo superfused rat dorsal medulla in a calcium-dependent fashion. The depolarizing alkaloid, veratridine, also evokes a large increase in efflux of labelled GABA. These data strongly suggest release from a neurotransmitter pool in this region.

There is considerable pharmacological evidence that the inhibitory amino acid GABA, or a similar substance, is an important neurotransmitter in regions of the dorsal medulla such as the dorsal column nuclei[2-4]. A logical step in the positive identification of GABA as a neurotransmitter in this region would be the demonstration of its neuronal release from intact (i.e. in vivo) tissue. There are, however, conflicting reports as to whether high potassium stimulation does[5] or does not[6] increase the efflux of labelled GABA from an in vivo dorsal medulla preparation. We have reinvestigated this problem using veratridine, electrical and high potassium stimuli, all 3 of which have in common a depolarizing action on neuronal tissues. A part of these data has been published previously in abstract form[7].

Materials and methods. Adult rats were anaesthetized with a 1% chloralose–10% urethane solution (8 ml kg^{-1} i.p.) and the dorsal surface of the medulla oblongata exposed. Approximately 2 h before the start of the experiment the animal was given an i.p. injection of amino-oxyacetic acid (20 mg kg^{-1}) to reduce GABA catabolism. A small acrylic superfusion chamber (internal volume 30 µl) was sealed in place concentric with that part of the cuneate nucleus providing the maximum ipsilateral forepaw evoked potential. Any GABA pools were labelled via their reuptake systems by 60 min exposure of the pial surface to cerebrospinal fluid (C.S.F.) containing either [1-^{14}C] labelled GABA (specific activity 2 µCi ml^{-1}, molar concentration 4.1×10^{-5} M) or [2,3-^{3}H] GABA (specific activity 10 µCi ml^{-1}, molar concentration 1.8×10^{-7} mM).

In control experiments the non-neurotransmitter amino acid L-[G-^{3}H] leucine (specific activity 10 µCi ml^{-1}, molar concentration 4×10^{-5} mM) was used. After a 60-min labelling period of closed cycle superfusion with the isotope (total volume 400 µl) the isotope was removed and superfusion continued at 50 µl min^{-1} with surrogate C.S.F. All solutions were buffered with Tris to pH 7.4 and osmolalities matched before use at 315 mOsm kg^{-1} H_2O. 5-min fractions of superfusate were collected serially in glass vials containing 6 ml Multisol II (Intertechnique Ltd) and 0.1 ml distilled H_2O and radioactivity estimated by liquid scintillation spectrometry. Counting efficiency was constant for ^{14}C isotopes but quench correction was routinely carried out for ^{3}H isotopes.

Chromatographic analyses were carried out on thin layer cellulose plastic plates using standard ascending chromatography.

Results and discussion. The figure, A, shows the spontaneous efflux of [1-^{14}C] GABA from the in vivo superfused dorsal medulla. When plotted semi-logarithmically this efflux consists of 2 or more phases for which lines of best fit have been computed by the least squares method. Although

the in vivo preparation has too many uncontrollable parameters to ascribe any biological significance for these different phases, the first, fast phase, but not the later, slower phases, can be reproduced by superfusion of non-neuronal tissue and is therefore likely to reflect extracellular washout of isotope. We have therefore attempted to evoke an increase in labelled GABA efflux during one of the later, slower phases.

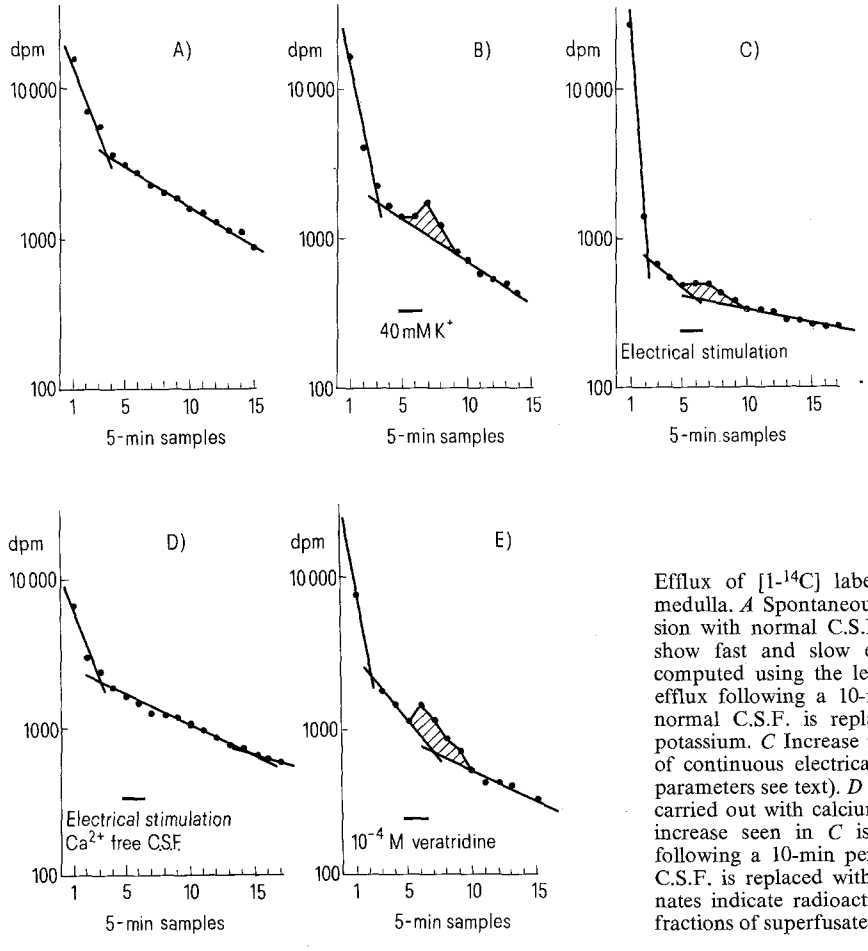
The figure, B, shows the evoked increase in efflux of label effected by changing from normal to high (40 mM) potassium C.S.F. for a 10-min period during one of the slow phases. This increase was consistently seen in 12 experiments, the mean increase in efflux of label being 32.8% ($p < 0.001$ in a paired t-test) for [1-^{14}C] GABA and 21.1% ($p < 0.001$) for [2,3-^{3}H] GABA. Chromatographic analysis of the superfusate at this time in the efflux slope indicated that the major part of the label remained associated with unmetabolized GABA, confirming a previous observation[5].

The efflux of labelled GABA could also be increased by electrical stimulation of the pial surface through bipolar silver electrodes insulated except at the tips and inserted concentrically with the superfusion chamber (figure, C). Stimulus parameters were 0.1 msec duration rectangular pulses, 1.5 mA, 400 Hz, the total stimulation lasting 10 min. An increased efflux was seen consistently in 6 experiments (mean increase 16.7%, $p < 0.001$). Computer-averaged cuneate evoked potentials were recorded using the stimulating electrodes both before and after each experiment. No abnormality in the electrical activity of the superfused region could be detected after the stimulating period, indicating that the stimulating current did not produce a lesion.

Both the potassium and electrically evoked increases in efflux were calcium-dependent and could be virtually abolished either by removal of calcium from the C.S.F. ($CaCl_2$ being replaced by $MgCl_2$) or by the addition of 10^{-3} M EDTA to calcium-free C.S.F. (figure, D).

The efflux of L-[G-^{3}H] leucine was not altered in any way by potassium or electrical stimulation. This, coupled with the observation that the increase in labelled GABA efflux is calcium-dependent, suggests that the evoked release of GABA is not due to alterations in extracellular space or to some other nonspecific mechanism such as changes in brain metabolism. However it is conceivable that both potassium and electrical stimulation could evoke an increased efflux of labelled GABA stored in a glial rather than a neuronal pool. It is difficult to distinguish between these 2 possible sources of release in the intact preparation. However one possible approach is by use of the depolarizing veratrum alkaloid, veratridine. Veratridine is thought to depolarize excitable tissues by preventing closure of the sodium channel. There is no evidence for it having any marked effect on inexcitable glial membranes and it has recently been reported to release labelled GABA in vitro from neuronal, but not from glial tissue[8].

In 5 experiments we changed the superfusion medium to one containing 10^{-4} M veratridine for a 10-min period at the same point on the efflux slope as previously used for potassium and electrical stimulation. This consistently evoked increases in efflux of label (figure, E) similar to those obtained with potassium and electrical stimulation (mean increase 30%, $p. < 0.01$). As with the other types of stimulation, veratridine had no effect on labelled leucine efflux.



Efflux of [1-^{14}C] labelled GABA from the superfused dorsal medulla. *A* Spontaneous GABA efflux during continuous superfusion with normal C.S.F. Data are plotted semilogarithmically to show fast and slow efflux components. Lines of best fit are computed using the least squares method. *B* Increase in GABA efflux following a 10-min period (horizontal bar) during which normal C.S.F. is replaced with one containing high (40 mM) potassium. *C* Increase in GABA efflux following a 10-min period of continuous electrical stimulation (horizontal bar, for stimulus parameters see text). *D* A similar experiment to that shown in *C* but carried out with calcium-free superfusate throughout. The evoked increase seen in *C* is abolished. *E* Increase in GABA efflux following a 10-min period (horizontal bar) during which normal C.S.F. is replaced with one containing 10^{-4} M veratridine. Ordinates indicate radioactivity (dpm), abscissae indicate serial 5-min fractions of superfusate.

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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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