

## INTRA- AND EXTRACELLULAR ANALYSIS OF MECHANISMS IN MOTOR CONTROL

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Our notions as to how stretch and contraction influence the excitability of motoneurones derive from electromyographic studies, from monosynaptic testing, and from observations of single-fibre responses in afferent and efferent rootlets. Lately our group at the Nobel Institute has added systematic studies on stretch (Granit, Kellerth and Williams, 1964<sup>20, 21</sup>) and contraction (Granit, Kellerth and Szumski, 1965<sup>19</sup>) by the intracellular approach and these are the ones that will be taken up below. Our preparation has been the cat under nembutal. Conventional microcapillaries filled with 2 M potassium citrate have been used as electrodes. It was, of course, necessary to pay special attention to fixation of the animal and its leg, even though, with the exception of semitendinosus (for stretch alone), only ankle muscles were used for stretch and contraction. In review- in this work figures will not be inserted but lantern slides will be presented at the Congress Lecture.

### *Contraction*

In contraction considerable interest attaches to the silent period of Hoffman (1919)<sup>26</sup> because it is sometimes maintained that this phenomenon is wholly due to cessation of activity in the spindle primaries which are unloaded during shortening of the extrafusal fibres producing the so-called 'pause' by which spindles are identified. The author has advocated a slightly different view (Granit, 1955<sup>18</sup>). If contraction is elicited from the peripheral nerve a prominent contribution to the silent period would obviously come from after-hyperpolarization and recurrent inhibition in motoneurones but in the work to be reviewed the appropriate ventral roots have been cut (L7 and S1 for the ankle extensors), and the peripheral stump of either of them stimulated, and so the physiological effects of contraction will condition the motoneurones before the latter are conditioned by a direct spike. (The so-called early discharge will be separately discussed below.) By varying extension with a constant stimulus, by further varying stimulus strength and duration at a given extension, it is possible to separate the effects of tension-sensitive receptors from those arriving from receptors responding merely to changes of length.

It had been shown long ago by monosynaptic testing (Granit, 1950<sup>17</sup>; Hagbarth and Naess, 1950<sup>24</sup>) that in extensor muscles contractile tension causes autogenetic inhibition, best marked when contraction is added to stretch. It has always seemed meaningless to the present author (*e.g.* Granit, 1955<sup>18</sup>) to exclude this inhibition from playing a role in the silent period. However, recently it has been shown (Granit *et al.*, 1964<sup>21</sup>) that autogenetic inhibition during stretch more often than not coincides with motoneurone depolarization and this observation raises a relevant question: what about the behaviour of the motoneurone membrane when subjected to impulses arising from autogenetic contraction?

To this question the experiments provided a perfectly definite answer. When gastro-

cnemius-soleus motoneurons or popliteal synergists were stimulated by isometric contractions elicited in the gastrocnemius-soleus muscles from a cut ventral root there arose a postsynaptic hyperpolarizing response. If the motoneurons were discharged repetitively by current injected from the tip of the micro-electrode, this phase of hyperpolarization during contraction was found to correspond to a silent period in the discharge. Now, to what extent is this postsynaptic inhibition dependent on tensile stress? It appeared that in all gastrocnemius-soleus motoneurons the amount of hyperpolarization increased when the muscle was extended from zero to 15 mm and, similarly, it increased when the size and rate of rise of tension was augmented by varying stimulus strength or stimulus duration (single shock *versus* brief tetani). The inhibitory postsynaptic response consisted of two phases: (i) an early one gently sloping towards (ii) an abruptly developing trough of hyperpolarization coinciding with the peak of contraction. The first phase was always present and strictly dependent on the rising tension for its appearance. It was absent in slack muscle and increased when the muscle was extended. The second phase was found in four cells. In those it could actually be seen also when the muscle was lying slack unhooked from the myograph. This effect also had the appearance of a 'trough' of hyperpolarization on top of contraction. This trough deepened considerably when more tension was produced in contraction. The early phase of slowly increasing tension-sensitive hyperpolarization must be due to the activity of tendon organs which accelerate early in contraction (Jansen and Rudjord, 1965<sup>29</sup>). The trough of hyperpolarization on top of contraction should be a combined effect of the pause in the discharge of spindle primaries and of tendon organs. The 'pause' would be relatively more important, the greater the background discharge from spindle primaries. There is thus real inhibition of a postsynaptic nature during the silent period. It is likely that cessation of spontaneous activity from the primary spindle organs in slack muscle contributes to the trough of hyperpolarization. The organs were denervated on the motor side but some activity may have remained. Afterhyperpolarization can be excluded in principle because many of the gastrocnemius-soleus motoneurons (and nearly all synergists) did not fire a spike when contraction was elicited from the peripheral stump of the ventral root. For the early phase of contraction recurrent inhibition cannot be excluded because surrounding motoneurons may have been discharged by this contraction. When motoneurons firing to contraction were struck by the micro-electrode, afterhyperpolarization succeeding this spike figured very largely in the intracellular records early in contraction. This effect could, of course, always be obtained by firing the muscle from the peripheral nerve instead of from the ventral root.

By monosynaptic testing a transient rise of autogenetic motoneurone excitability was found early in the relaxation phase of contraction (Bianconi, Granit and Reis, 1964<sup>2, 3</sup>). This moment corresponds to the time of appearance of the so-called myotatic appendage or 'myotatic hump' of Ballif, Fulton and Liddell (1925)<sup>1</sup>, and, no doubt, this rise of excitability was caused by the bursts from phasically sensitive spindle primaries, recently studied by Granit and Van Der Meulen (1962)<sup>23</sup>. Its intracellular equivalent is a brief hump of depolarization which serves as a kind of timing-device putting an end to the silent period whose length, of course, also will depend to some degree on other determinants of motoneurone excitability. This 'timing-device' explains why the silent period, even in synergists, is so definitely dependent upon the contraction time of the muscle which causes it (Merton, 1950<sup>37</sup>, 1951<sup>38</sup>). For this reason the duration of the silent period can give but little information on the actual amount of contractile inhibition that preceded its termination.

Stimulation of ventral roots often produces a back-response (Lloyd, 1942<sup>33</sup>; Leksell, 1945<sup>32</sup>) from the terminals. Hunt and Kuffler (1951)<sup>28</sup> called this the 'early response'

and they found it to consist of discharges from both spindle and tendon organs. Granit, Pompeiano and Waltman (1959)<sup>22</sup> analyzed it in an ephaptic component and a slightly later discharge early in contraction. In some motoneurons the effect of such discharges were visible, in others not, as is understandable because back-firing is restricted to a limited number of afferents whose projections may or may not terminate on the motoneuron which happens to be located by the micro-electrode. When present, the effect of the back-response is seen as a brief wavelet of hyperpolarization or depolarization, considerably earlier and of briefer duration than any of the synaptic events so far described. The sign of the effect apparently depends upon whether tendon organs or spindle primaries happen to be dominant at the particular motoneuron isolated. The average effect, elucidated by monosynaptic testing, is always excitatory.

By monosynaptic testing Bianconi *et al.* (1964)<sup>2</sup> found a late inhibition in extensors. This succeeded the myotatic hump and extended into the period of relaxation. Because this event in flexors had opposite sign, being excitatory in character, they ascribed it to spindle secondaries. These organs are inhibitory in extensors and excitatory in flexors (Lloyd, 1943<sup>34, 35</sup>; Brock, Eccles and Rall, 1951<sup>6</sup>; Hunt, 1954<sup>27</sup>; Laporte and Bessou, 1959<sup>31</sup>) and, besides, are lacking the phasic properties of the spindle primaries (Cooper, 1959<sup>7</sup>, 1961<sup>8</sup>; Lundberg and Winsbury, 1960<sup>36</sup>; Harvey and Matthews, 1961<sup>25</sup>; Bianconi and Van Der Meulen, 1963<sup>4</sup>). In some gastrocnemius-soleus motoneurons one finds a similar late inhibition by the present technique. When a steady rate of firing is maintained by transmembrane stimulation, the silent period may extend far into the relaxation phase or end up in a long-lasting reduction of discharge frequency. Often it is possible to demonstrate wavelets of inhibitory or hyperpolarizing 'activation noise' during the relaxation phase. It is generally impossible to find a maintained hyperpolarization of the cell. This fact brings up a point of considerable importance. It concerns the nature of the intracellular indicators of inhibition. Clearly, with well-synchronized stimuli such as those used by Eccles and his colleagues (Eccles, 1957<sup>10</sup>, 1964<sup>11</sup>; Brock, Coombs and Eccles, 1952<sup>5</sup>) their IPSP, familiar to all students of nervous activity, is a most reliable index of inhibition. But we shall now see that static muscle stretch, which produces an asynchronous barrage of impulses of opposite sign, E+I, is a more complex affair meaning that changes of membrane potential or absence of such changes cannot always be unequivocally interpreted.

### *Stretch*

Often activation noise, consisting of wavelets of potential greatly varying in size, is the only visible effect of muscle stretch (Granit *et al.*, 1964<sup>20</sup>). When antagonist muscles such as semitendinosus or tibialis anterior influence popliteal motoneurons hyperpolarizing wavelets tend to predominate. With autogenetic combinations, as, for instance, when popliteal motoneurons are activated by stretch of triceps surae, the noise may be mixed in character, de- or hyperpolarizing, but perhaps predominantly depolarizing. If the depolarizing wavelets of activation noise are large and/or the cell sufficiently depolarized, they may give rise to spikes. Very likely some external synchronizing influence contributes to the production of the wavelets characterizing this particular mode of firing. It may well represent one of the ways in which motoneurons normally are activated.

Alternatively considerable changes of steady level of potential may arise as a consequence of autogenetic stretch, say, depolarizations from 5 to 15 mV, and the motoneuron may then discharge the way a sense organ is fired by its generator potential. Evidence was obtained showing that the large currents needed for such depolarizations are delivered by interneurons. Firing on top of substantial waves of depolarization was

described for respiratory motoneurons by Eccles, Sears and Shealy (1962)<sup>13</sup>, Sears (1964)<sup>39</sup> and confirmed by Gill and Kuno (1963)<sup>16</sup>. From the pyramidal end motoneurons may likewise be stimulated to act in this manner (Landgren, Phillips and Porter, 1962<sup>30</sup>).

Consider next the case in which the activation noise to stretch is of a very modest order or too mixed (E + I) to be readily interpretable. From the point of view of the tasks expected from the motoneurone this may be called sheer dissipation of energy in the form of noise. How is the experimenter then to know whether the stretch impulses caused excitation or inhibition? Changes of membrane potential may be negligibly small or even absent. The method we (Granit *et al.*, 1964<sup>21</sup>) devised for this purpose was based on firing the motoneurone by current injected from the tip of the micro-electrode. Against this background, effects of muscle stretch were easily demonstrated. Excitation, of course, appeared as an acceleration of the steady rate of discharge, inhibition as a de-celeration or complete suppression of it. Since the firing was maintained by an injected current such effects of stretch receptors must have reached the cell membrane itself and hence must have been postsynaptic. This point is of considerable significance because the postsynaptic nature of an inhibition established by such means seems reasonably secure.

Applying this method it turned out that (i) in general the autogenetic effects of pull were excitatory and thus led to an acceleration of the rate of discharge, quite independent of whether the membrane potential was increased, decreased or uninfluenced. Into this category fell the effects of stretch of triceps surae on popliteal, of semitendinosus on hamstring, and of tibialis anterior + extensor digitorum longus on peroneal motoneurons. (ii) With antagonistic combinations of flexors against extensors or vice versa, the firing rate was reduced or the discharge to the injected current wholly suppressed. There was thus postsynaptic inhibition. Some hamstring motoneurons proved to be synergic with those of the triceps surae. (It should be recalled that semitendinosus inserts on the tibia and apparently some of its motoneurons help to fix the leg in synergy with the ankle extensors.) Of particular interest was the strong inhibition, often with hyperpolarizing activation noise, that semitendinosus pull exerted on many popliteal motoneurons because with this combination it would not have seemed unreasonable to expect the leading inhibitory effects to be presynaptic (Eccles, Eccles and Magni, 1961<sup>12</sup>; Eccles, 1964<sup>11</sup>). With brief stretch lasting 10 msec. only and hence probably approximating to synchronous shocks, Devanandan, Eccles and Yokota (1964)<sup>9</sup> have recently shown that the group of phenomena, interpreted to indicate presynaptic inhibition, can be elicited by such stretches. However, our method is likely to be restricted to the postsynaptic inhibitions, often in such cases indicated also by inhibitory activation noise or maintained membrane hyperpolarization.

From the point of view of motor control our major finding may be described as the fact that (transmembrane) depolarization was needed to make the motoneurone itself translate into action the peripheral message produced by stretching muscles. And this held good for both excitation and inhibition. Actually 'artificial' depolarization made the motoneurone act in accordance with what one was entitled to expect from classical reflexology, i.e. there was autogenetic excitation and antagonistic inhibition. Whence do the necessary membrane currents arise which in the normal life of an animal produce the depolarization needed in the motoneurons for their role as interpreters of stretch impulses? Apparently from a large variety of biasing systems, supraspinal, local and spinal, acting through the mediation of interneurons, or across the gamma-loop. "Unless stimulation in any one system is highly synchronous, it is likely to be rather ineffective without support from some other system" (Granit, 1955<sup>18</sup>).

The sensitivity of the method of using transmembrane firing as a background for indicating postsynaptic effects produced by 'natural' stimuli deserves further study. Nevertheless, in combination with observations on activation noise, on monosynaptic responses and on changes of membrane potential, it has already proved its value because the method is clearly sensitive enough to demonstrate definite effects of excitation or inhibition by muscle stretch in cases when other methods fail to provide an unequivocal answer. When, for instance, the monosynaptic EPSP diminishes or is uninfluenced by stretch causing little if any change of the membrane potential, the discharge produced by transmembrane stimulation may be accelerated or stopped. To the micro-electrode tip events in a motoneurone may be 'remote' until depolarization brings them within reach of observation whatever their sign. This also means that diffuse or non-specific afferents, by producing the general excitation (depolarization) which here has been imitated by transmembrane stimulation, may throw a variety of specific inhibitory and excitatory patterns into operation.

In the work referred to we (Granit *et al.*, 1964<sup>21</sup>) also made a number of measurements of the relation between size of the monosynaptic test EPSP and the amount of change of membrane potential during stretch. When these results were plotted out, separately for autogenetic and antagonistic combinations, they showed that there was no definite relationship between these two quantities. A reduction of EPSP was a fairly regular effect of autogenetic stretch and the most common change of membrane potential, if anything at all was seen, was a maintained depolarizing response. Whenever in such cases autogenetic stretch was combined with transmembrane firing, the rate of discharge was increased. The reduction of the EPSP with autogenetic stretch was attributed to the following causes: (i) direct interference between excitatory and inhibitory activation noise; (ii) engagement of synapses making them non-responsive to a testing volley hitting the same synapses and (iii) membrane leaks through the conductances created by the impulse barrage arriving at the excitatory synapses and reducing the current flow of the constant EPSP.

With antagonistic combinations, in the majority of cases, there was likewise a diminution of the size of the EPSP the amount of which appeared independent of whether the membrane potential was augmented, diminished or uninfluenced. As already pointed out above, some hamstring motoneurones proved synergic with the popliteal ones. Clearly a diminution of the size of the EPSP as an indicator of inhibition must be used with extreme caution. Many inhibitions which are highly potent when tested on a firing motoneurone are 'remote' with respect to other indicators such as size of EPSP, change of average membrane potential and activation noise. I refer to the original paper (Granit *et al.*, 1964<sup>21</sup>) for a discussion of 'remoteness', this term being used in a wider sense than by Frank and Fuortes (1957)<sup>15</sup> and Frank (1959)<sup>14</sup> whose results have been explained by presynaptic inhibition (Eccles, summary 1964). Ours deal with postsynaptic inhibitions.

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