Effects from the Pyramidal Tract on Primary Afferents and on Spinal Reflex Actions to Primary Afferents

It has recently been shown that activity in the pyramidal tract facilitates a variety of spinal reflex paths to motoneurones by excitatory action at an interneuronal level[1]. Observations made in this connection raised the question whether the pyramidal tract may also influence primary afferents. Recent investigations have revealed that depolarization of presynaptic terminals of primary afferents in all likelihood constitutes a physiological reflex action mediated by interneurones and causing presynaptic inhibition[2].

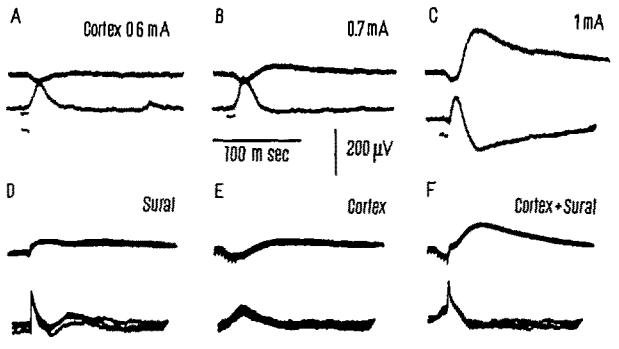

Fig. 1. The upper traces were recorded from a dorsal root filament in lower L6. The filament was cut 15 mm from the dorsal root entry zone and placed on two electrodes, one close to the entry zone and the other on the cut end. Upwards deflection denotes negative of the central electrode. The lower traces were recorded from the dorsal root entry zone in L7 against an indifferent electrode in the muscle. The sensorimotor cortex was stimulated at the indicated strengths in A–C. Records D–F are from another experiment. Record D shows the effect of stimulation of the sural nerve at a strength giving a submaximal dorsal root potential. The sensorimotor cortex was stimulated in E, the strength being at threshold for evoking a dorsal root potential. F shows spatial facilitation on combined stimulation with the same strength as those used in D and F respectively. The records in D–F consist of superimposed traces. The anaesthesia was Chloralose.

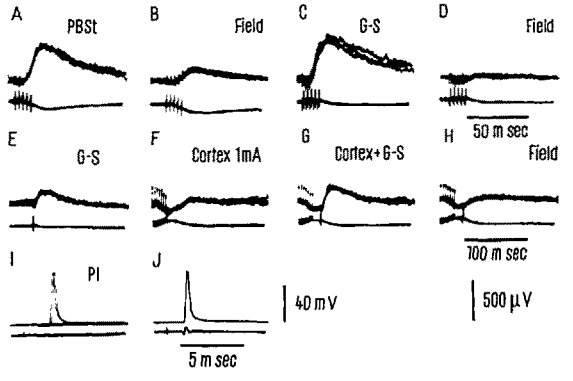

Fig. 2. The upper traces were recorded with a microelectrode inserted through the dorsal column to the intermediary region of the spinal cord in L7 and the lower traces from the dorsal root entry zone. The intracellular records in A, C, and E–G were obtained from a group I afferent fibre from plantaris and the corresponding records in B, D, and H are the extracellular field potentials recorded after withdrawal of the microelectrode from the fibre. The effect of a train of group I volleys in the nerve to posterior biceps-semitendinosus (BSt) and in the nerve to gastrocnemius-soleus (G-S) in A and C respectively. The differences between these records and the field potentials in B and D give the depolarizations evoked. Record E shows the depolarization evoked by a single group I volley in the G-S nerve, F the effect of stimulation of the sensorimotor cortex and G the spatial facilitation resulting in combined stimulation of cortex and the G-S nerve (field in H). The plantaris nerve was stimulated at threshold for the axon in I and at maximal group I strength in J (low amplification). Throughout the intracellular recording the membrane potential was 70 mV. All records consist of superimposed traces. The animal was anaesthetized with Nembutal.

Stimulation of the sensorimotor cortex (cat) gives a large depolarizing dorsal root potential (Figure 1, C) and correspondingly a positive dorsal horn potential after an initial negative wave. In animals with a hemisectioned cord, bilateral effects are evoked from cortex. The depolarizing dorsal root potential requires stronger cortical stimulation than the negative dorsal horn potential (Figure 1, A and B) but is evoked from the same cortical area as the negative dorsal horn potential and the effects to motoneurones and to interneurones of spinal reflex arcs. Dorsal root potentials cannot be evoked from cortex after section of the pyramid, hence this effect to primary afferents is mediated by the pyramidal tract.

Intraspinal threshold measurement[3] in cutaneous and Ia afferents (recording from peripheral nerves) revealed increased excitability on cortical stimulation in the former but not in the latter system of afferents. Correspondingly it was found with intracellular recording preterminally from axons that stimulation of the sensorimotor cortex evoked a depolarization in cutaneous and also in Ib afferents, but not in Ia afferents.

The interaction between the effects evoked in primary afferents from cortex and from peripheral nerves was investigated in detail. Of particular interest is the finding of spatial facilitation. Cortical stimulation at a strength subliminal for effect on primary afferents markedly facilitates submaximal negative dorsal root potentials evoked from group I afferents, cutaneous afferents (Figure 1, D–F), high threshold muscle and joint afferents. Intracellular recording from primary afferents revealed facilitation from cortex of effects from different afferent systems to cutaneous afferents. There was also facilitation from cortex of the reflex depolarization from group I muscle afferents on to group I muscle afferents, as is illustrated in Figure 2, E–H, for a low threshold fibre from plantaris, which received depolarization from group I fibres of posterior biceps-semitendinosus (A–B), gastrocnemius-soleus (C–D) and also from the pretibial flexors, quadriceps, flexor digitorum longus and anterior biceps-semitendinosus (not illustrated).

Stimulation of the sensorimotor cortex may through depolarization of primary afferents give inhibition of spinal reflex arcs. Our results strongly suggest that this effect is exerted via the interneurones mediating spinal reflex actions to primary afferents. The sensorimotor cortex may utilize the interneuronal network of spinal reflex arcs to distribute depolarization to primary afferents or else the physiological significance of our findings may be that the sensorimotor cortex can mobilize spinal reflex actions to primary afferents. This suggestion falls in line with postulates regarding the functional significance of

[1] A. Lundberg and P. Voorhoeve, Exper. *17*, 46 (1961).—A. Lundberg and P. Voorhoeve, Acta physiol. scand., in press.—A. Lundberg, U. Norrsell, and P. Voorhoeve, Acta physiol. scand., in press.

[2] J. C. Eccles, Proc. Roy. Soc. B *153*, 445 (1961).—J. C. Eccles, R. M. Eccles, and F. Magni, J. Physiol. (Lond.) *159*, 147 (1961).—J. C. Eccles, F. Magni, and W. D. Willis, J. Physiol. (Lond.) *160*, 62 (1962).

[3] P. D. Wall, J. Physiol. (Lond.) *142*, 1 (1958).

actions from the pyramidal tract on reflex paths to motoneurones[1].

Zusammenfassung. Es konnte gezeigt werden, dass Überleitungen in spinalen Reflexsystemen, die für die präsynaptische Depolarisation gewisser primärer Afferenzen verantwortlich sind, von der sensorisch-motorischen Grosshirnrinde über die Pyramidenbahn gefördert werden kann.

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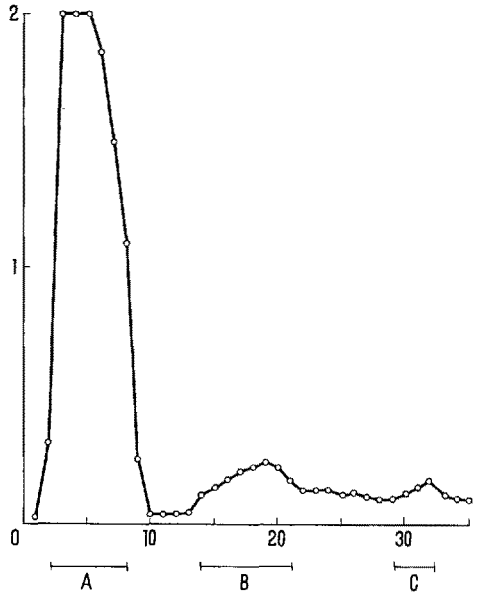
Isolation of a Hypothalamic Peptide with TRF (Thyreotrophin Releasing Factor) Activity *in vitro*

A hypothalamic factor activating adenohypophysial acid phosphatases *in vitro* was prepared from acetic acid extracts of bovine hypothalami by high voltage electrophoresis and its TSH-releasing activity was demonstrated *in vitro*[1,2]. A review of previous work on the relationship between adenohypophysial acid phosphatase and TSH secretion and on the characteristics of the activating factor was published elsewhere[3].

The active factor was now further purified by gel filtration on a Sephadex G-25 column and the probable peptide character was demonstrated by a combined electrophoretic and chromatographic analysis.

Fractions HH_{18+1} and HH_{20+1}, prepared by high voltage electrophoresis from lyophilized acetic acid extracts of 85 and 120 bovine hypothalami respectively, were dissolved (170 mg and 700 mg respectively) in 1 cm³ 0.225% NaCl and applied to a Sephadex G-25 column (1.7 cm × 12.0 cm), saturated with 0.225% NaCl. The column was eluted in an automatic fractions collector with 0.225% NaCl and 35 and 50 fractions respectively (3 cm³ each) were collected (flow = 6 cm³/h). The extinction of these fractions was measured at 280 mμ and three peaks were found, as shown for fraction HH_{18+1} in the Figure. Separation of the individual peaks in gel filtration of fraction HH_{20+1} was less distinct, probably because a larger amount of material was used. The fractions giving the individual peaks were pooled to give subfractions $HH_{18+1,A}$ (85 mg after lyophilization and subtraction of NaCl), $HH_{18+1,B}$ (45 mg), $HH_{18+1,C}$ (15 mg), $HH_{20+1,A}$ (410 mg), $HH_{20+1,B}$ (150 mg) and $HH_{20+1,C}$ (68 mg).



Gel filtration of fraction HH_{18+1} on a Sephadex G-25 column. Abscissa: Number of fraction; ordinate: extinction at 280 mμ. Fractions corresponding to individual peaks of extinction were pooled as indicated to give fraction $HH_{18+1,A}$, $HH_{18+1,B}$, and $HH_{18+1,C}$.

Tab. I. Acid phosphatase activity in μg phenol liberated by 1 mg adenohypophysial tissue in 30 min. Concentration of the fractions 10 μg/cm³.

1st experimental series				
Group	I	II	III	IV
Number of tests	24	24	24	18
Fraction	0	$HH_{18+1,A}$	$HH_{18+1,B}$	$HH_{18+1,C}$
Acid phosphatase activity	3.0 ± 0.06	3.3 ± 0.09	3.1 ± 0.09	3.6 ± 0.08[a]
2nd experimental series				
Group	I	II	III	IV
Number of tests	18	18	18	18
Fraction	0	$HH_{20+1,A}$	$HH_{20+1,B}$	$HH_{20+1,C}$
Acid phosphatase activity	2.5 ± 0.18	3.1 ± 0.13	2.1 ± 0.12	3.3 ± 0.09[a]

[a] Comparison with group I in Fisher's *t*-test $p < 0.01$.

Acid phosphatase activity was measured in rat adenohypophysial homogenate[3] in the presence of subfractions A, B, and C in concentration of 10 μg/cm³. The results are shown in Table I. Slight elevation of activity was found in the presence of the A fractions, but more important elevation of activity occured in the presence of the C fractions. Both C fractions ($HH_{18+1,C}$ and $HH_{20+1,C}$) were therefore used to test their effect on TSH release *in vitro* and for electrochromatographic analysis.

For electrochromatographic analysis of the C fractions, the method described by JIRGL[4] was used. 1 mg of fraction $HH_{18+1,C}$ or $HH_{20+1,C}$ was dissolved in 1 cm³ H_2O and applied to the middle of a 12 × 30 cm strip of Whatman 3 paper (native specimen). The same amount of the fractions was dissolved in 0.5 cm³ H_2O, 0.5 cm³ 6*N* HCl was added and the specimen was heated at 115°C for 24 h in a sealed beaker (hydrolyzed specimen). The hydrolyzate was then dried *in vacuo* at 100°C, dissolved in 1 cm³ H_2O

[1] V. SCHREIBER, A. ECKERTOVÁ, Z. FRANC, J. Kočí, M. RYBÁK, and V. KMENTOVÁ, Exper. *17*, 264 (1961).
[2] V. SCHREIBER, J. Kočí, A. ECKERTOVÁ, and V. KMENTOVÁ, Physiol. Bohemoslov. *10*, 417 (1961).
[3] V. SCHREIBER, Acta Univ. Carol. Medica (Prague) *7*, 33 (1961).
[4] V. JIRGL, Exper. *15*, 235 (1959).