Intracellularly Recorded Responses of Red Nucleus Neurones During Antidromic and Orthodromic Activation

The red nucleus (RN) of the cat, in its so-called magnocellular portion, includes a number of giant-sized neurones which send their axons into the spinal cord as the rubrospinal fibres[1–3]. The main source of its afferent connections has been shown histologically to be the contralateral cerebellar nuclei; JANSEN and JANSEN[4] assumed it to be in the nucleus interpositus, while BRODAL and GOGSTAD[5] maintains that it is in the dentate nucleus. Very recently RINVIK and WALBERG[6] proposed another afferent connection from the ipsilateral cerebral cortex to RN. Electrical activities in RN have recently been investigated by APPELBERG[7] and MASSION and ALBE-FESSARD[8] with microelectrode technique. In view of the large gaps still remaining in our knowledge of the performance of RN, the present work is an attempt to record from RN neurones with the intracellular technique. The region of RN was explored systematically with glass microelectrodes, and neurones impaled therein were identified by antidromic activation from the spinal cord through the rubrospinal fibres. It was further revealed that, in these cells, stimulation at the three loci, i.e. the contralateral nucleus interpositus (IP), the ventrolateral nucleus of the ipsilateral thalamus (VL) and the ipsilateral sensorimotor cortex (SM), induced excitatory postsynaptic potentials (EPSPs) monosynaptically.

Cats were anaesthetized with pentobarbitone sodium or chloralose. The head was fixed on a stereotaxic frame and the spinous process of the C_2 vertebra was also rigidly clamped. After craniotomy the cerebral cortex was partly sucked out so as to expose the left superior colliculus through which a 3 *M* KCl- or 2 *M* NaCl-filled microelectrode (electrical resistance of 15–25 MΩ) was inserted vertically. In order to stimulate the rubrospinal tract, the C_2 vertebra was laminectomized and an enamel-coated silver wire was placed on the dorso-lateral surface of the C_2 segment on the right side. A pair of enamel-coated metal electrodes held in parallel at an interval of 2 mm was inserted stereotaxically into the regions of the right IP and of the left VL, respectively. The left SM and sometimes the intermediate part of the right anterior lobe of the cerebellum (AC) were also exposed by craniotomy and four silver wire electrodes were laid at intervals of 2 mm on each of their surfaces.

Figure A shows examples of the potential changes recorded extracellularly with a microelectrode which, after being inserted vertically to a depth of 10 mm from the surface of the superior colliculus, was withdrawn in 100–250 μ steps. In response to stimulation of the C_2 segment, there appeared a small positive deflection that was followed by a large negativity. The negativity was localized within a depth of 8 to 10 mm with the maximum at a depth of 9.05 mm. By repeating similar trackings on the same transverse plane at lateral intervals of 250 μ, it was ascertained that the prominent negative potential changes, such as those of more than 250 μV in amplitude, were generated only within a limited area about 2 mm across. By later histological examination, this area was proved to conform with the magnocellular part of RN. Therefore, these potential changes would be attributable to the electric field generated in RN.

When a cell was impaled within the RN region, there appeared suddenly a resting potential of −50 to −60 mV and spike potentials of 60 to 70 mV in response to stimulation of the C_2 segment. Their rising phase showed an inflection at 23 to 33 mV above the base line (Figure B). When two successive stimuli were applied to the C_2 segment at short intervals, the second spike was usually blocked, leaving only a small component spike (Figure C). In some cases, the blockage occurred also at the inflection. On the analogy of the spike potentials evoked in cat's motoneurone by stimulating the ventral root[9], these spike potentials obtained in the RN neurones may be assumed to be induced antidromically through the rubrospinal tract. The falling phase of the spike potentials was followed, either with or without an interposed positive dip (Figure D), by an after-hyperpolarization of 5 to 10 mV in amplitude and lasting for 50 to 70 msec. In Figure E the strength of shock stimuli was just at threshold for the axon of the impaled cell, the spike failing at about half trials. With this procedure of the 'threshold-straddling', it has not been possible to detect any postsynaptic potential changes which might occur through the recurrent axon collaterals.

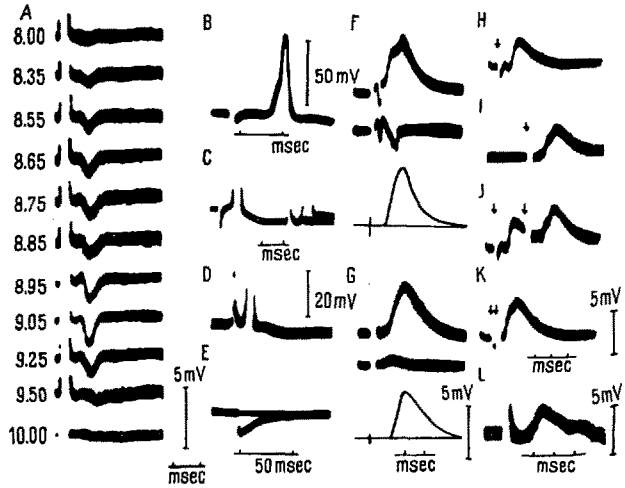

Fig. A. An electric field recorded along a track passing through RN under stimulation at the dorsolateral surface of the C_2 spinal segment. Each record was formed by superposition of about 60 faint traces. B to L, potential changes recorded intracellularly from RN neurones. B, Spike potentials elicited antidromically from the C_2 segment. C, Response to double shocks given to the C_2 segment. D, Falling phase of the antidromic spike potential. E, After-hyperpolarization following an antidromic spike potential. F and G, EPSPs induced by stimulation of IP and VL, respectively (the uppermost traces); the middle traces indicate extracellular records taken just outside the cell and the lowermost traces represent differences between the middle and uppermost records. H and I, the control EPSPs evoked by stimulation of IP and VL, respectively. J and K, EPSPs caused by combined stimulation of both nuclei. L, EPSP induced by stimulating SM. A and F to L, by A.C. amplification, time constant of the amplifier being 0,002 and 2 sec, respectively. B to E, by D.C recording. Voltage scale of 20 mV applies to C to E.

[1] C. VON MONAKOW, Neurol. Cbl. *1910*, 724.
[2] O. POMPEIANO and A. BRODAL, J. comp. Neurol. *108*, 225 (1957).
[3] A. HINMAN and M. B. CARPENTER, J. comp. Neurol. *113*, 61 (1959).
[4] J. JANSEN and J. JR. JANSEN, J. comp. Neurol. *102*, 607 (1955).
[5] A. BRODAL and A. C. GOGSTAD, Anat. Rec. *118*, 455 (1954).
[6] E. RINVIK and F. WALBERG, J. comp. Neurol. *120*, 393 (1963).
[7] B. APPELBERG, Acta physiol. scand. *51*, 356 (1961).
[8] J. MASSION and D. ALBE-FESSARD, Electroenceph. clin. Neurophysiol. *15*, 435 (1963).
[9] J. S. COOMBS, D. R. CURTIS, and J. C. ECCLES, J. Physiol. *139*, 198 (1957).

In Figure F, in response to the shock stimuli applied to IP, there occurred an excitatory postsynaptic potential (EPSP) with notches on its summit (the uppermost traces of Figure F). With a microelectrode just outside the cell soma, these notches were found to be due to field potentials occurring around the impaled cell (the middle traces). As difference between the intracellular and extracellular records the net EPSP was reproduced in the lowermost traces of F and found to be of simple shape, as those seen in cat's motoneurones[10]. As measured by the time intervals between the shock artifact and the foot of the EPSPs, the latency of the EPSPs was as short as 0.7 to 1.0 msec. Hence these EPSPs should have been induced monosynaptically. As shown in Figure G similar EPSPs were generated by stimulating VL with a latency of 1.0 to 1.2 msec. Concerning the fibre connection responsible for these VL-induced EPSPs, the following observation revealed a marked occlusion phenomenon between the VL and IP stimulation. As shown in Figure J, when the time interval for stimulating these two loci successively was relatively long, the respective EPSPs summated on each other without significant change in their size (controls in Figures H and I). However, when the interval was shortened to less than 1.0 msec, the response to the second stimulus decreased markedly, down to one third in the case of Figure K. Such a reduction of the succeeding EPSP as this was seen with either sequence of VL-IP or IP-VL stimulation. This occlusion phenomenon could be explained by assuming that the axons from IP innervate RN and VL commonly, and that stimulation at VL causes impulses to fire back to RN. In some cases shock stimuli were applied to AC. With relatively weak intensity, the AC stimulation did not induce postsynaptic potentials in the RN neurones, but it suppressed by about 20% the EPSP induced by excitation of IP. The suppression followed the AC stimulation immediately and lasted for several ten-milliseconds. The AC stimulation produces inhibitory postsynaptic potentials monosynaptically in IP neurones[11], and hence decreases the excitability of IP neurones. With relatively strong AC stimulation EPSPs were produced in the RN neurones. However, these EPSPs occurred with a latency comparable to those produced by the direct IP stimulation, and hence appeared to be caused by current spreading to IP from AC.

In Figure L EPSPs were generated in response to the shock stimuli applied to SM with a latency of about 1.2 msec. With a distance of 40 mm from SM to RN, it is likely that these EPSPs also are induced monosynaptically.

In conclusion, the RN neurones receive two monosynaptic excitatory connections, the one from IP and the other from SM. The former involves VL as the common target and is under inhibitory control by AC.

Résumé. Chez le chat anesthésié au Nembutal ou au chloralose, les réponses évoquées dans les neurones du noyau rouge par les stimulations antidromiques et orthodromiques ont été étudiées au moyen de microélectrodes intracellulaires.

N. TSUKAHARA*, K. TOYAMA**, and K. KOSAKA*

**Department of Physiology, Faculty of Medicine, University of Tokyo (Japan), and **Department of Physiology, Faculty of Medicine, University of Nagoya (Japan), June 11, 1964.*

[10] J. S. COOMBS, D. R. CURTIS, and J. C. ECCLES, J. Physiol. *130*, 374 (1955).

[11] M. ITO, M. YOSHIDA, and K. OBATA, sent to Experientia.

Inulin Clearance and Renal Lymph

In calculating the clearance of any substance excreted in the urine from the customary formula, relatively large errors occur whenever not all of the substance is carried away in the urine and *via* the renal vein. The error is the greater the more of the substance has undergone decomposition, been stored up by the kidney, and carried off in the renal lymph. Some workers[1-3] see the possibility that clearances calculated from the usual formula do not yield true values of the quantity of plasma cleared, because they fail to take into account the amount of substance transported in the lymph. Evidence has recently been produced that the amounts of *para*-amino-hippuric acid and inulin transported by the renal lymphatics are not large enough to invalidate the clearance values corresponding to the renal plasma flow and the glomerular filtration rate respectively[4-5].

As intravenous inulin is known to appear in readily measurable concentrations in the renal lymph[4,6-8] experiments were carried out to examine its influence on interpretation of clearance data obtained from the general formula.

Methods. From dogs urine was collected, after laparotomy, from the left ureter and lymph from one of the hilar lymph vessels in the left kidney. The clearance periods and the lymph collection periods were of the same duration. Inulin was determined by the procedure of LITTLE[9]. Inulin clearance was calculated from the well-known quotient

$$\frac{\text{amount of inulin excreted in the urine per min (mg/min)}}{\text{concentration of inulin in the plasma (mg/ml)}}$$

and

$$\frac{\text{amount of inulin in the renal lymph per min (mg/min)}}{\text{concentration of inulin in the plasma (mg/ml)}}$$

[1] P. BÁLINT, in *Probleme des Nierenkreislaufes*, Symposium über die Methodik der Bestimmung der Nierendurchblutung (Verlag der ungarischen Akademie der Wissenschaften, Budapest 1962), p. 20.

[2] M. FÖLDI, Orv. Hetil. *105*, 104 (1964).

[3] B. JOSEPHSON, personal communication, 2nd Intern. Congress of Nephrology (Praha 1963).

[4] M. G. BULL and P. METAXAS, Clin. Sci. *23*, 515 (1962).

[5] G. SZABÓ, M. PAPP, and S. MAGYAR, Exper. *18*, 128 (1962).

[6] E. S. BREED and A. E. DURMONT, Surg. Forum *11*, 103 (1960).

[7] A. KAPLAN, M. FRIEDMAN, and H. E. KRUGER, Am. J. Physiol. *138*, 553 (1943).

[8] M. PAPP, Orv. Hetil. *104*, 2070 (1963).

[9] M. LITTLE, J. biol. Chem. *180*, 747 (1949).