Intracellular Analysis of Antidromically and Synaptically Activated Nucleus Reticularis Tegmenti Pontis Neurons

The nucleus reticularis tegmenti pontis (NRTP) is a precerebellar nucleus which is located dorsal to the pontine nuclei proper and is bounded dorsally by the raphe nuclei and pontine reticular formation. The major afferent input to this nucleus is from the deep cerebellar nuclei via the brachium conjunctivum[1–5]. The output of the NRTP is directed almost exclusively to the cerebellum[6]. The importance of this precerebellar nucleus in terms of cerebellar operation has been elucidated so far mainly by anatomical studies. The present report deals with an electrophysiological study of the NRTP neurons by antidromic and synaptic activation.

Cats were anesthetized with a combination of surital (30 mg/kg) and chloralose (80 mg/kg). During recording, muscular movements were eliminated by injection of gallamine triethiodide and the animal was artificially ventilated. Bipolar stimulating electrodes with an inter-electrode distance of 1 mm were inserted stereotaxically in the nucleus interpositus (IP), decussation of brachium conjunctivum (DBC), brachium pontis (BP) and cerebral peduncle (CP). Stimuli were brief current pulses of 0.05–0.1 msec. Glass microelectrodes (3 *M* KCl, 15–20 *M*) were used and conventional recording methods employed.

Because the NRTP is so closely adjacent to the other pontine nuclei, much care was taken in identifying the recording site. First, the NRTP neurons were identified by their antidromic responses to stimulation of the BP. The nucleus was approached from the ventral surface of the pons and the recording electrode was positioned stereotaxically. The recording sites were also identified histologically and in some cases, identification was by reference to extracellularly injected dye spots[7].

The antidromically activated spike had an IS-SD inflection on its rising phase and was followed by an after-hyperpolarization lasting around 20 msec. Double shock tests showed SD and IS spike refratoriness to be around 1.5 msec and 0.8 msec, respectively. The latencies of the antidromically activated spikes following BP stimulation varied from 0.2 msec to 1.0 msec ($N = 32$ mean 0.5 mesc). This latency is similar to the latencies of antidromically activated pontine nuclear cells[8,9]. The estimated conduction velocities of NRTP neurons ranged from 10 m/sec to 50 m/sec (mean, 20 m/sec).

The Figure illustrates some synaptically activated phenomena in NRTP cells. Figure A shows IP induced EPSPs with a latency (determined by comparing the intracellular records with the extracellular controls) of 1.2 msec. Considering the conduction distance and synaptic delay, and the relatively constant latencies regardless of stimulus strength, and their graded nature, these depolarizing potentials were considered to be monosynaptic EPSPs. Figure B–D shows samples of monosynaptic EPSPs (latency 0.8 msec duration 10 msec) induced by DBC stimulation. The latency of the IP induced EPSPs ranged from 0.9 msec to 2.2 msec ($N = 27$ mean 1.37 msec) and the duration from 4 msec to 24 msec (mean 10 msec). DBC induced EPSPs had a latency range of 0.7 msec

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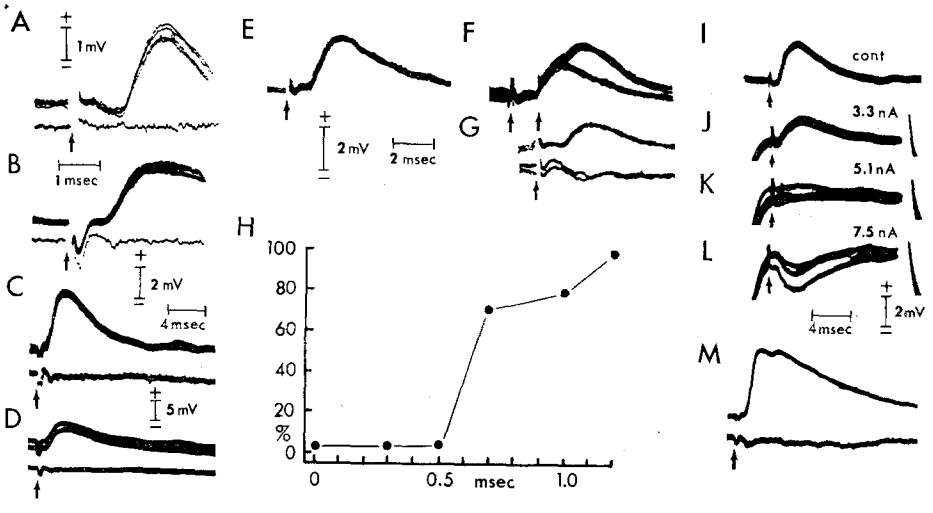

Intracellular recordings of EPSPs in NRTP cells. A) IP induced EPSP. B) DBC induced EPSP. C) EPSPs same as in B except with slower sweep speed. D) DC records of EPSPs as in C. E) and F) sample records of collision test between EPSPs induced by DBC (conditioning) and IP (test) stimulation at 0 msec interstimulus time intervals (E) and 1.2 msec (F). Control DBC response is superimposed in E and F. G) control test response. H) a plot of % changes in the amplitude of test EPSPs at various inter-stimulus interval. Ordinate: % of changes from control test responses as 100% Abscissa: interstimulus time interval. I–L) effects of depolarizing current on IP induced EPSPs; intensity of applied current indicated on upper right corner of each record. M) CP induced EPSPs. Bottom traces of records in A–D, G and M are corresponding extracellular control responses. Voltage calibration of 1 mV applies to A, 5 mV to D and 2 mV to B, C and E–M. Time calibration of 1 msec applies only to A and B.

to 1.4 msec ($N = 42$, mean 0.95 msec) and the duration varied from 5 msec to 30 msec (mean 10 msec). In some NRTP neurons, EPSPs were induced following IP as well as DBC stimulation. Collision experiments illustrate (Figure E–H) that IP induced EPSPs were blocked by conditioning DBC stimulation at stimulus intervals of less than 0.5 msec. A partial recovery was observed at 0.7 msec and full recovery at 1.2 msec inter-stimulus intervals. Considering the latencies of DBC and IP induced EPSPs and the results of collision experiments, NRTP cells are monosynaptically activated by axons of IP cells via the brachium conjunctivum. This conclusion is in agreement with anatomical observations[1–4]. Conduction velocity of the IP axons impinging on the NRTP cells was estimated by measuring differences in latencies between IP and DBC induced EPSPs and the distance between stimulation sites. The conduction velocity thus calculated from 6 intracellularly recorded NRTP cells ranged from 25 M/sec to 50 M/sec (mean 33 M/sec). At times, the stimulation of IP resulted in antidromic firing of the NRTP neurons followed by monosynaptic EPSPs or synaptically activated spikes. This could be interpreted as supportive evidence for a reciprocal pathway between the IP and pontine nucleus as suggested by TSUKAHARA and BANDO[9].

Some of the IP induced EPSPs were reversed by application of depolarizing currents through the recording microelectrode (Figure I–L). Figure I is a control record of an IP induced EPSP. By successive increases in depolarizing current the amplitude of the EPSPs decreased (Figure J). The transmembrane potential was almost at its reversal potential at 5.1 nA (Figure K) and was reversed at 7.5 nA intensity (Figure L). These results would indicate that some excitatory synaptic terminals of IP axons are located on or near the soma of the NRTP cells[10].

Figure M shows an example of monosynaptically induced EPSPs following CP stimulation. The latency of CP induced EPSPs varied from 0.7 msec to 1.5 msec ($N = 10$, mean 1.1 msec) and the duration from 7–26 msec (mean 13 msec). This average latency of 1.1 msec is similar to that of pontine nuclear cells activated by CP stimulation[9]. Direct cortical inputs to the NRTP cells have been observed by anatomical methods[11–14].

In conclusion, the present data show, in agreement with previous anatomical observations, that the NRTP neurons receive direct excitatory inputs from the cerebellar nucleus via the brachium conjunctivum and from the cerebral peduncle. Some electrophysiological characteristics of the NRTP cells are different from those of the LRN cells[15,16] indicating functional differences between these precerebellar nuclei in terms of cerebellar operation.

Résumé. On a enregistré les potentiels electriques mono- et poly-synaptiques (EPSPs) des cellules du nucleus reticularis tegmenti pontis en stimulant le nucleus interpositus (NI), le brachium conjunctivum (BC) et le pédoncule cérébelleux. Les expériences de collision ont montré que des axones des cellules NRTP activent leurs neurones via BC. Quelques potentiels postsynaptiques exitateurs induits par le nucleus interpositus ont été inversés par un courant dépolarisant appliqué au voisinage de la microélectrode de dérivation, ce qui indique que les terminaisons synaptiques d'axones NI se trouvent près du sommet des cellules NRTP.

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Influence of Altered Sterol Composition on (Na^+, K^+) ATPase Activity of Cardiac Sarcolemma

It was shown that in experimental myopathy induced by 20.25-diazacholesterol the activity of (Na^+, K^+) ATPase in membranes of different tissues is increased[1–3]. It was supposed that this increase is due to the incorporation of desmosterol in the different membranes; 20.25-diazacholesterol inhibits desmosterol reductase and therefore desmosterol is accumulated in the treated animals[4].

In this study we tried to find a correlation between the replacement of cholesterol by desmosterol and the increase of specific (Na^+, K^+) ATPase activity in cardiac sarcolemma. 4 groups of male Wistar-rats were studied over a period of 30 days. Group A served as control and received 0.2 ml water by an oesophageal cannula, group B was treated with 10 mg 20.25-diazacholesterol dihydrochloride in 0.2 ml water, group C corresponds to group B but additionally had 2% cholesterol in the standard diet, group D was treated with a suspension of 20 mg triparanol in 0.2 ml water – this substance is also known to inhibit cholesterol biosynthesis at the step of interconversion from desmosterol to cholesterol[5]. All groups of rats were treated daily with the above-mentioned substances. After intervals indicated in the Table, the animals were sacrificed by aortic puncture, cardiac sarcolemma was then prepared according to DIETZE and HEPP[6] and sterol analysis was performed as described earlier[1] in a varian 1400 gaschromatograph equipped with a 3% OV-17 column.

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