Time course of excitatory and inhibitory states of bulbar respiratory modulated neurons

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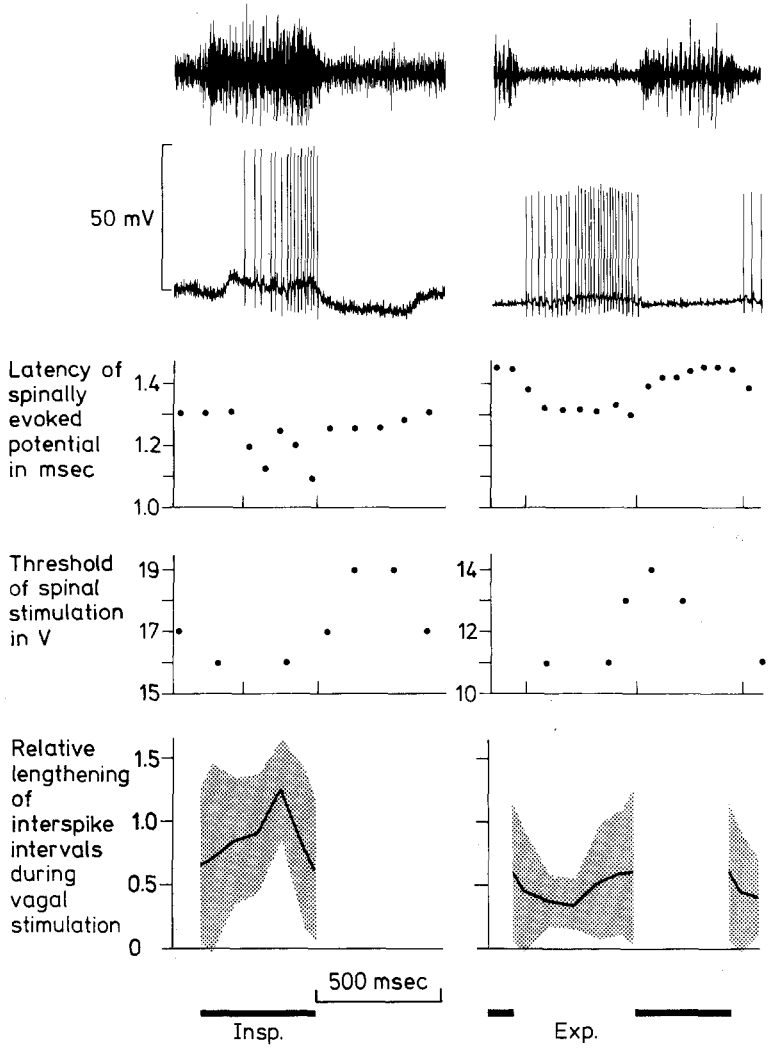
Summary. In respiratory modulated neurons of rabbits, vagally mediated inhibition is not bound to resting membrane potential oscillations. Latency of spinally evoked antidromical spike invasion, however, is shorter and threshold voltage is lower during the shift of membrane potential towards depolarization accompanying burst discharge.

In rabbits, bursting activity of some bulbar respiratory modulated neurons (RMN) is bound to inspiration (I neurons), of others to expiration (E neurons). It is still unknown whether bursting activity is an inherent cellular property of RMN. The importance of mutual interaction between I and E neurons for burst generation, most probably inhibitory in nature, has been stressed[1]. Rhythmic shifts of resting membrane potential towards de- or hyperpolarization and concomitant increase or decrease of unit excitability should result.

In rabbits anesthetized with 1.1 g urethane/kg b.wt, intra- or extracellular recordings were made from RMN located in the bulbar reticular formation in the region of the obex, using micropipettes. As can be seen from the figure, in I and E neurons resting membrane potential was shifted towards depolarization shortly before (in the I neuron) or during burst discharge (in the E unit), and was shifted towards hyperpolarization during the silent period in both cells. This time course of resting membrane potential is essentially similar to that described for cats[2-4] and is compatible with the theory of mutual inhibition. Depolarization of spinal motoneurons due to synaptic excitatory action has been shown to facilitate axon-soma transmission and to shorten the delay of antidromic spike propagation into the soma[5]. In the figure, the uppermost diagrams show oscillation of latency of action potentials evoked antidromically by spinal shocks (delivered at 10 pps at C2). In both units, latencies were shorter during burst discharge than during the silent period. The hypothesis was brought forward that latencies of antidromic spike invasion closely reflect excitability of RMN in cats[6].

The following diagrams exhibit fluctuations of threshold voltage of spinal stimuli throughout the respiratory cycle. In the I neuron, threshold was lower at the end of the silent period and during the first half of spike discharge than during the first half of the interburst interval. An essentially



Typical oscillation of central excitatory or inhibitory state during inspiration and in I neurons on left side and during expiration and in E units on right side. From the top, the traces are: phrenic nerve activity showing phase relation of cell discharges; intracellular recordings from units (unretouched playback from tape recording). Inspiration and expiration are marked again at the bottom. The uppermost pair of diagrams shows latency of action potentials antidromically evoked by spinal stimuli (each dot represents a single pulse). The following diagrams exhibit the time course of threshold voltage of spinally evoked action potentials. The next diagrams illustrate lengthening of interspike intervals caused by single vagal stimuli delivered at 10 pps (the duration of the lengthened interspike interval was related to the duration of the preceding uninfluenced control interspike interval; mean values and SD are shown).

similar time course of threshold voltage was encountered in the E unit. It is quite likely that with increasing stimulus strength, recruitment of additional descending or ascending fibres occurred, excitation of which facilitated antidromic spike invasion in RMN. If facilitation is synaptic in nature and 0.5 msec are allowed for synaptic transmission, these facilitatory fibres must conduct faster than the axons emerging from the facilitated RMN. If the facilitatory action potentials are supposed to travel antidromically, they may travel along axons originating from faster conducting RMN which send an axon collateral to the facilitated unit. The mean latency of antidromic spike invasion of RMN was found to be 1.27 msec and the range ($\bar{X} \pm SD$) was 0.65–1.89 msec[7]. On the other hand, they may travel along axons originating from other types of bulbar neurons, the discharge of which is not modulated with respiration. Mean latency of antidromic spike invasion in this type of cell was found to be 0.47 msec and the range was 0.22–0.72 msec[7] which leaves enough time for facilitation of all RMN. The facilitating spikes, however, may well travel orthodromically along spinal ascending fibres. The time course of excitability determined from threshold voltages was somewhat different from the time course of latencies of antidromic spike invasion; threshold voltage was lower during the later part of the silent period.

In addition to the above mentioned mutual inhibitory mechanism, inspiratory activity is inhibited by excitation of pulmonary stretch afferents (Hering-Breuer inflation reflex). Single electrical pulses delivered to the vagal nerves immediately entail lengthening of one interspike interval in more than $^1/_3$ of all RMN tested[8]. Close examination then revealed that lengthening was more pronounced in I than in E neurons, and in the former cell type mainly in the later part of burst discharge; lengthening was modest in the middle part of bursting activity of E units (figure). Variability in the lengthening was high at respiratory phase transitions. Lengthenings of interspike intervals, and thus the inhibitory state of RMN, apparently do not reflect the time course of the resting membrane potential of I and E neurons, but rather parallel that of the central inspiratory 'off-switch' mechanism[9,10].

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Effect of sympathectomy on platelet aggregation and blood coagulation in rats

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Summary. It is shown that the ability of blood platelets to aggregate in partially and completely sympathectomized rats is significantly lower than in intact animals. The blood clotting system of sympathectomized rats is hyperactive. The sympathectomy-provoked changes may be due to the increased content of adrenaline in the blood.

It is known that in addition to involving plasma components, blood clot formation also involves blood cells, predominantly platelets. The adhesiveness platelets and their ability to aggregate, stimulated by collagen, thrombin, adrenaline, ADP and some other substances, can favour thrombosis in blood vessels[1,2]. An indispensable regulator of intravascular blood coagulation is the nervous system; the dysfunctions of the sympathetic or parasympathetic nervous systems cause changes in hemostasis[3].

In previous experiments we demonstrated an experimental model of heart cavity thrombosis, developed in rats with a congenital lack of sympathetic peripheral innervation[4–6]. Under stress conditions the sympathectomized animals perished due to extensive thrombosis in the auricular cavity.

The aim of the present work is to investigate the aggregation of blood platelets in sympathectomized rats.

Materials and methods. Sympathectomy was performed on mongrel rats by injecting newborn animals with guanethidine, which causes irreversible degeneration of sympathetic ganglia cell organelles[7,8]. The experimental animals were divided into 2 groups. The 1st group included rats with partial sympathectomy (PSR), caused by injecting newborn rats with guanethidine daily for 2 weeks. The stellate ganglia of these animals contained only 25% of the normal amount of neurons. The 2nd group included animals with total sympathectomy (TSR) caused by a 4-week administration of guanethidine; in this case the content of neurons in the stellate ganglia was only 0.5%. In both experimental groups the ADP-induced aggregation was determined in 1.5, 2.5 and 4-month-old animals; the thrombin-induced aggregation was measured in 1.5 and 2.5-month-old animals of group 2 alone.

Blood samples were collected from the jugular vein, using 3.8% sodium citrate solution (9:1). Platelet aggregation was assayed according to Born[10]. The aggregation was induced by ADP ("Reanal") or thrombin ("Sigma") at final concentrations of 10 mkM and 0.1μ, respectively. The recalcification time, thrombin time and the blood adrenaline content of experimental animals were determined.

Results and discussion. As can be seen from the table, the platelet aggregation in sympathectomized animals is significantly decreased. Sympathectomy results in a total blocking of the thrombin-provoked aggregation and a marked inhibition of the ATP-induced aggregation. The number of platelets in whole blood samples of intact and sympathectomized animals in practically the same.

The degree of aggregation induced by ADP or thrombin in TSR does not depend on age. However, in the case of PSR the ADP-provoked platelet aggregation is age-dependent, e.g. in 4-month-old rats the aggregation is statistically significantly higher than in animals from other age groups ($p < 0.001$).