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Changes in short-term potentiation of hypoglossal activity by modulators of nitric oxide production in the rabbit

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Abstract. Stimulation of the superior laryngeal nerve (SLN) causes a potentiation of hypoglossal nerve activity persisting after cessation of stimulation. The mechanism of this phenomenon is uncertain. We investigated a potential role of the nitric oxide (NO) pathway in modulation of the after-effects of SLN stimulation on phrenic and hypoglossal activity in rabbits. L-Arginine, a substrate for NO synthesis and NG-Nitro-L-Arginine (L-NNA) an inhibitor of NO synthase (NOS), were administered systemically. L-Arginine and L-NNA alone caused small changes in respiratory activity. During pre-treatment with NO precursor the amplitude and duration of hypoglossal potentiation evoked by SLN stimulation were reduced. Systemic NO synthase inhibition partially reversed these effects of L-Arginine. The results showed that interference with NO production by NO substrate and NOS inhibitor modulates the effects of SLN stimulation on hypoglossal activity. Nitric oxide might be a negative modulator of the transmission of short-term potentiation (STP) in hypoglossal activity.

Key words: short-term potentiation, hypoglossal nerve activity, phrenic nerve activity, nitric oxide

INTRODUCTION

A gradual disappearance of respiratory changes elicited by afferent stimulation was demonstrated for the first time by Gessell and co-workers (1942) for carotid sinus nerve stimulation. This observation was confirmed by studies of Eldridge and Millhorn (1986). Post--stimulatory behaviour of the respiratory activity was described in rabbits following stimulation of the vagus nerve, mesencephalic reticular formation and rostral pons (Budzińska et al. 1978). Stimulation of the superior laryngeal nerve (SLN) depresses or inhibits phrenic nerve activity. However, the respiration-related hypoglossal nerve activity is potentiated and this potentiation persists after cessation of stimulation (Jiang et al. 1991). Post-stimulatory effects of various inputs to the central generator of breathing can be considered to be a form of respiratory plasticity. The time constant of these phenomena depends on the input and can range from few seconds to several minutes. It seems that the underlying mechanism and anatomical sites of the phenomena with a longer time constant are better understood than for the short lasting effects. For instance, the long-term respiratory effect of carotid sinus nerve stimulation or hypoxia is presumably serotoninergic because it is blocked by methysergide, a serotonin antagonist, given either systemically or into the medullary raphe nuclei (Fregosi and Mitchell 1994, Bach and Mitchell 1996). Respiratory activity, both tonic and phasic, is modulated by raphe stimulation (Budzińska and Romaniuk 1995). Hypoglossal motoneurones have serotoninergic innervation coming from raphe medullary nuclei (Manaker and Tischler 1993) and several types of serotoninergic receptors are present in the hypoglossal nucleus (Okabe et al. 1997). Indeed, methysergide, a serotonin antagonist, mostly of 5HT1/5HT2 receptors, strongly reduces activity of hypoglossal neurones (Fenik et al. 1997). It seems, however, that 5HT1A agonists that are inhibitory do not make an important contribution to hypoglossal activity modulation (Douse and White 1996). Studies of Jiang and co-workers (1991) based on the effects of methysergide suggested that serotoninergic transmission is not necessary for the respiratory short-term potentiation of laryngeal origin. Methysergide is a broad-spectrum antagonist of serotonin receptors, however, one cannot exclude the engagement of the specific type of serotonin receptor in this phenomenon.

The SLN provides motor and sensory innervation of the larynx. Respiratory responses of laryngeal origin are processed in the structures involved in the inspiratory termination such as the solitary tract nucleus (Jiang and Lipski 1992, Mifflin 1993, Ranson et al. 1995, Takagi et al. 1995) and the parabrachial nuclei (Car et al. 1975, Ootani et al. 1995, Miyaoka et al. 1998). It was shown also that nitric oxide, NO, a putative intracellular messenger in the CNS, participates in all types of innervation of the larynx in the rat (Hisa et al. 1996 a, Robertson et al. 1998) and the dog (Hisa et al. 1996 b). Moreover, nitric oxide synthase-positive neurones and processes were detected in the nucleus of the solitary tract. There are suggestions that modulation of neuronal activity by NO in the brain stem is circumscribed to the NTS and the parabrachial nucleus (Dun et al. 1994, Torres et al. 1997). Nitric oxide is known to play a role in cardiorespiratory regulation (Rees et al. 1989, Gozal et al. 1996, Liu et al. 1996, Vitagliano et al. 1996, Teppema et al. 1997), in chemoreceptor function (Prabhakar et al. 1993, Trzebski et al. 1995), and in certain forms of synaptic plasticity (see for review Hawkins et al. 1998). It has been postulated that NO generated by eNOS in hippocampus acts as a retrograde messenger in the induction of memory (Dawson and Snyder 1994). Contribution of NO to neural plasticity and its presence in the structures processing respiratory information from SLN opens the question of a possible role of NO pathway in the modulation of hypoglossal and phrenic responses to this input, and in the potentiation of hypoglossal activity after cessation of the SLN stimulus.

Part of these results was presented in abstract form (Budzińska and Wojtal 1995).

METHODS

The experiments were carried out on 12 rabbits of either sex (2.5-3.8 kg of body weight). The animals were anaesthetised with a mixture of urethane with chloralose (1.2 g/kg and 51 mg/kg respectively). Following a tracheostomy rabbits were paralysed with a dose of 0.08 mg/kg of pipecuronium bromide (supplemental doses were administered every hour) and artificially ventilated with oxygen-enriched air. The femoral arteries and vein were cannulated for measurements of blood pressure, blood gases concentration and pH and for injections of agents, respectively. A respiratory gases meter, Respina IH 26, continuously monitored fractional concentrations of end-tidal CO_2 and O_2. Artificial ventilation was adjusted to keep blood gases at the level of 40-45 mm Hg

for CO_2 and 105-110 mm Hg for O_2. Rectal temperature was maintained at 37-38°C with a heating pad.

The animals were subjected to midcervical bilateral vagotomy. The C_4 rootlet of the phrenic nerve and hypoglossal nerve were cut and their central ends were placed on recording silver bipolar electrodes. The superior laryngeal nerve was prepared for electrical stimulation.

Phrenic and hypoglossal nerve activities were amplified, filtered (0.5-5.0 kHz) and integrated with time constant 70 ms. SLN stimulation consisted of a train of stimuli with 0.5 ms pulse duration and 50 Hz frequency. Strength of SLN stimulation was determined on a case by case basis as the threshold current that caused an inhibition of phrenic nerve activity. It ranged from 150-500 μA. The nerve activity along with the stimulus marker were displayed on line at the computer screen and stored for off-line analysis by an Adcjul Acquisition System. Blood pressure and remaining variables were also recorded on a Honeywell Omnilight 8 M36 recorder.

Changes in NO production were evoked by intravenous injection of L-Arginine (Sigma), a substrate for NO synthesis, at a dose of 300 mg/kg, and of NG-Nitro-L--Arginine, L-NNA (Sigma), an inhibitor of NO synthase, at a dose of 30 mg/kg. The L-Arginine was dissolved in 0.9% NaCl and the L-NNA in 0.1N HCl. The pH of both solutions was adjusted to physiological pH of the animal with 0.1N NaOH. L-NNA is a very potent NOS blocker administered systemically. Since L-NNA causes an inhibition of NOS activity which can not be reversed (Dwyer et al. 1991), we decided in the experimental protocol first to increase the production of NO by L-Arginine and then to decrease it by L-NNA.

The experiment was conducted as follows: Electrical stimulation of SLN started always in the expiratory phase and lasted 20 seconds. Once the strength of stimulation was determined the SLN was stimulated in the control. Next L-Arginine was given systemically and stimulation was repeated 8 and 30 minutes after injection. Thirty-five minutes after L-Arginine injection, L-NNA was administered. The SLN was stimulated again after 30 minutes. Control injections of equal volume of the vehicle were also given.

Calculations: The peak amplitude of integrated activity of phrenic and hypoglossal nerves was measured in the control and after L-Arginine and L-NNA treatment and the effect of SLN stimulation at these stages of the experiment was estimated. The induction of short-term potentiation evoked by SLN stimulation was calculated from phrenic and hypoglossal amplitude averaged during the first 30 seconds after cessation of stimulation. Data were calculated as percent value of the amplitude before stimulation and shown as mean ± SEM. The duration of the effect that expresses maintaining of the STP, was calculated as the time lapse from the end of stimula-

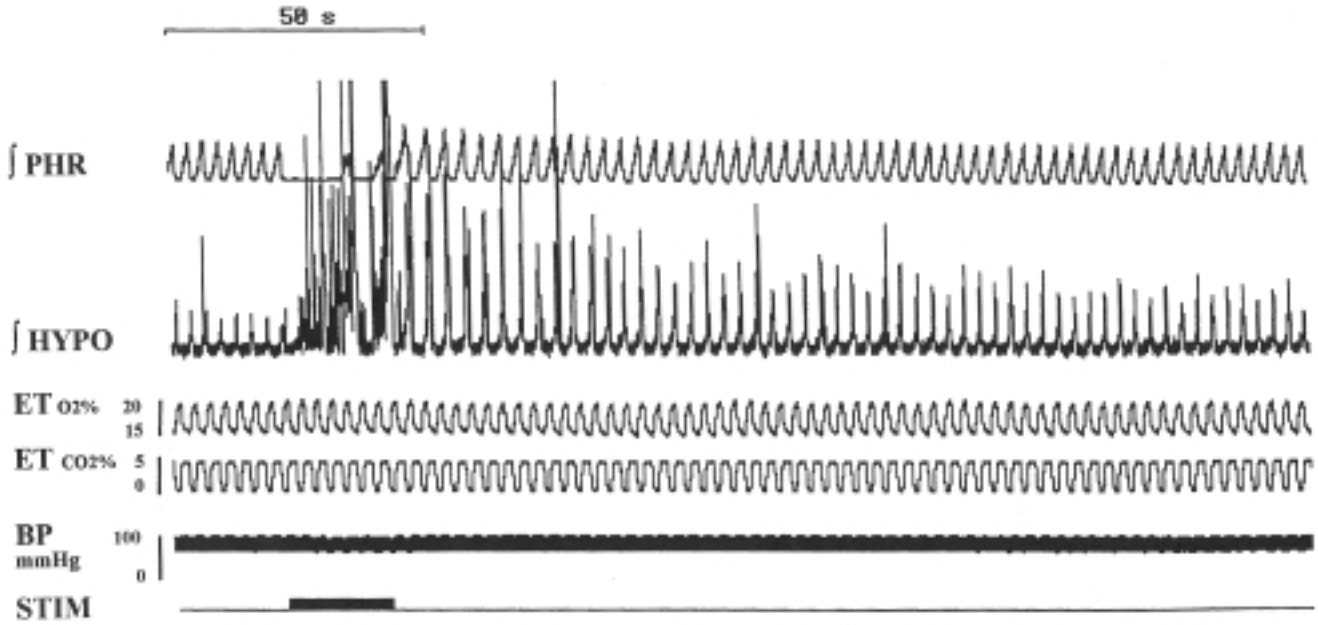

Fig. 1. Effect of SLN stimulation on phrenic and hypoglossal nerve activity in control conditions. Record from top to bottom: integrated phrenic nerve activity, integrated hypoglossal nerve activity, end- tidal O_2, end-tidal CO_2, arterial blood pressure, stimulus marker.

tion to the return to control amplitude. For hypoglossal nerve activity changes in the amplitude during SLN stimulation were also estimated. Statistical analysis of the effects during and after stimulation was performed by two-tail Student's paired *t*-test.

RESULTS

Effects of SLN stimulation during control

The onset of the stimulus trains to SLN activated tonic hypoglossal nerve activity (Fig. 1). This activity increased strongly with superimposed periodic bursts (tonic plus peak activity increased to 285% ± 53.6) corresponding to that of phrenic nerve. Tonic hypoglossal activity decreased immediately after termination of stimulation. Peak amplitude of the phasic activity was higher than before stimulation. Mean amplitude taken during first 30 seconds from the end of stimulation was 175.4% ± 23.85 (Fig. 2 control). The augmented hypoglossal amplitude returned to control values within 4.38 ± 1.04 minutes (Fig. 3). We could observe a decay of the response as long as 8 minutes.

Stimulation of SLN (Fig. 1) caused either an inhibition of phrenic nerve activity or a decrease in frequency along with a depression of the phrenic burst. The inhibitory effect differed from animal to animal. In some experiments, complete inhibition of phrenic nerve activity was achieved after one or two depressed phrenic bursts while in others the inhibition was immediate. Which of those two effects occurred apparently did not depend on the strength of stimulus because with increasing intensity of stimulation a progressive depression and lower rate of the phrenic bursts were seen instead of complete inhibition of this activity. After cessation of stimulation two effects, not related to the pattern of the response during stimulation, were observed. The phrenic amplitude either recovered immediately or it increased slightly (mean 107.7% ± 2.8) and then returned to the pretrain amplitude within 1.3 ± 0.2 minutes (Figs. 4 and 5 control).

Effect of SLN stimulation after administration of L-Arginine

Intravenous administration of L-Arginine diminished slightly the amplitude of hypoglossal (91.9% ± 3.3) and phrenic activity (96.5% ± 1.2). The respiratory timing did not change. Systemic application of L-Arginine caused 10-15 mm Hg decrease in arterial blood pressure that started soon after administration and stabilised at about 4 mm Hg less than the baseline after a period of 6-8 minutes. Changes in blood pressure are presented in Table I.

SLN stimulation elicited the same pattern of response in hypoglossal nerve activity as in control conditions (Fig. 2). The increase in hypoglossal amplitude was lower (223.3% ± 45.4). When stimulation ceased, the augmented hypoglossal amplitude declined rapidly to 123.7% ± 14.6 as calculated for the first 30 seconds. In addition, the duration of augmented hypoglossal activity shortened to 1.8 ± 0.6 minutes (Fig. 3). Thirty minutes after injection of L-Arginine we observed further attenuation of SLN stimulation effects. At that time, stimulation caused 185.0% ± 27.6 increase in hypoglossal amplitude. Furthermore, during 30 seconds after termination of stimulation the potentiated

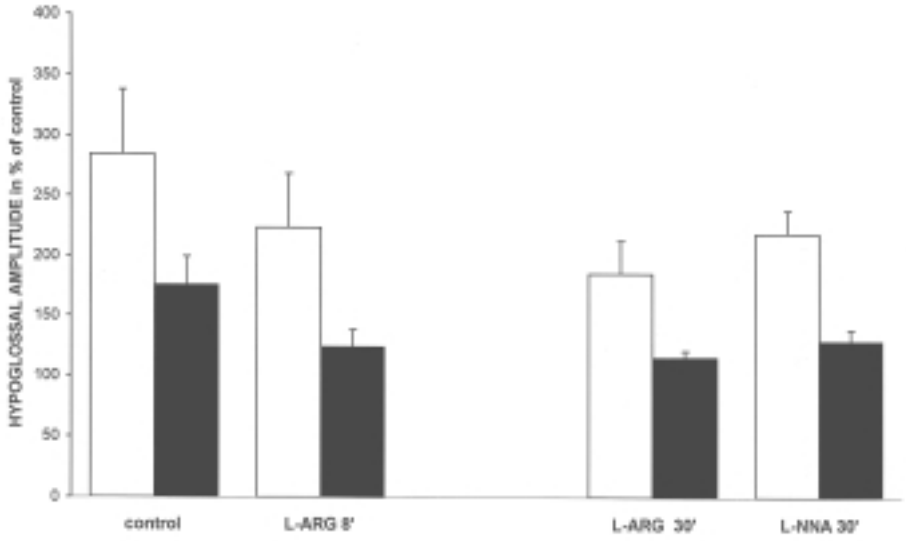

Fig. 2. Relative changes in amplitude of integrated hypoglossal activity during (white bars) and within 30 seconds after cessation of (grey bars) SLN stimulation in the control (n = 11) and following L-Arginine (n = 9) and L-NNA (n = 8) administration. Bars indicate means ± SEM.

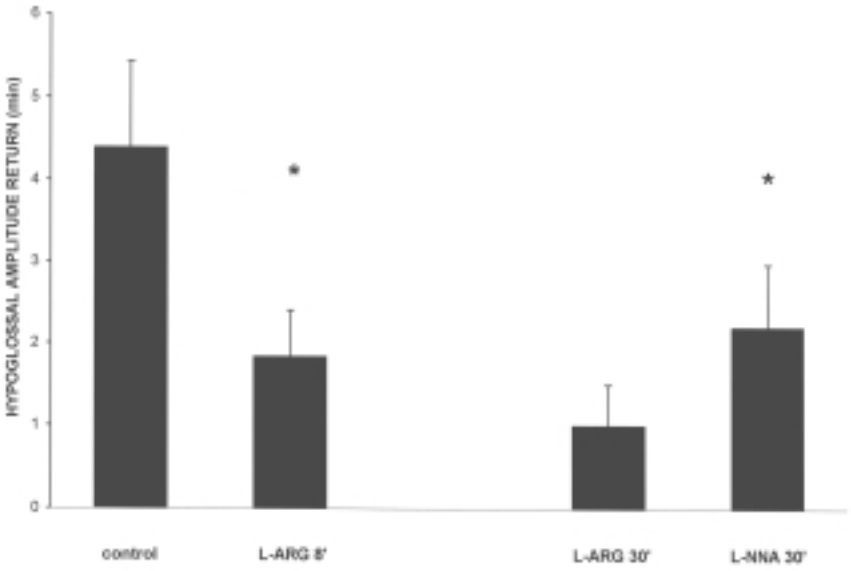

Fig. 3. Time of the decay of short-term potentiation of hypoglossal amplitude in the control and after L-Arginine and L-NNA administration. There is a significant difference tested with Student's paired *t*-test between duration of the effect in control and after L-Arginine ($P<0.01, n = 11$) and between the effects seen 30 minutes after L-Arginine and after L-NNA ($P<0.05$, $n = 8$) shown by asterisks. Bars indicate means ± SEM.

hypoglossal activity decreased to 115% ± 5.6 and the duration of the augmentation declined to 1.0 ± 0.5 minute. Changes in duration of the augmented activity were statistically significant ($P<0.01$ for eight minutes after L-Arginine and $P<0.05$ for thirty minutes after L-Arginine).

Eight minutes after L-Arginine, cessation of SLN stimulation caused only slight signs of potentiation (104.6% ± 2.6) in phrenic nerve activity lasting 0.9 ± 0.2 min. Thirty minutes after L-Arginine the effect was similar (104.5% ± 1.2) and it lasted only 0.6 ± 0.06 min (see Figs. 4 and 5 L-Arg bars).

Effect of SLN stimulation after administration of L-NNA

L-NNA itself caused an increase in amplitude of hypoglossal nerve activity up to 124.6% ± 16.9 and a smaller increase for phrenic nerve (105.4% ± 2.7). Blood pressure increased, reaching a value close to the baseline (Table I). Stimulation of SLN elicited the same pattern of response as in control and under L-Arginine (Fig. 2), yet tonic and phasic hypoglossal activities increased more (218.5% ± 19.6) than during stimulation after L-Arginine. Post-stimulatory hypoglossal amplitude decreased during

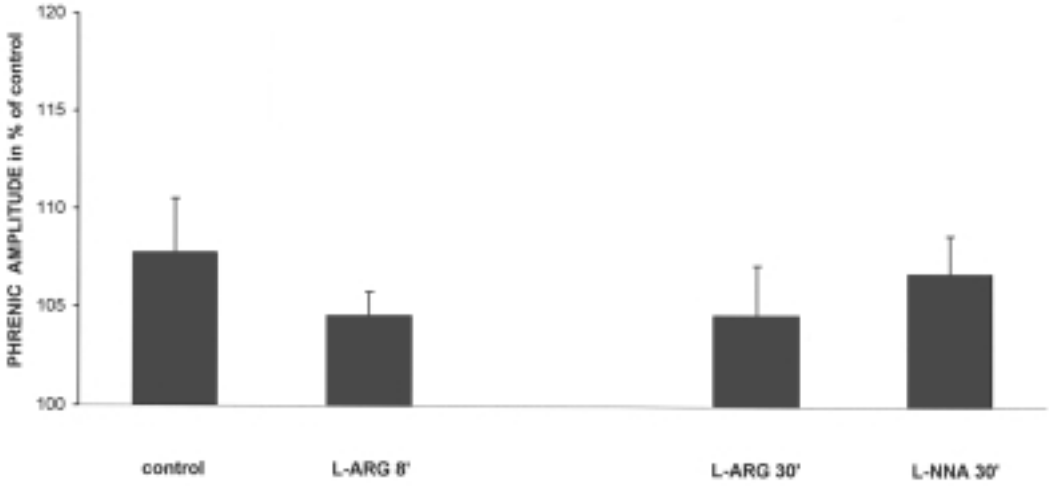

Fig. 4. Relative changes in amplitude of integrated phrenic activity within 30 seconds after cessation of SLN stimulation in the control ($n = 11$) and following L-Arginine ($n = 9$) and L-NNA ($n = 8$) administration. Bars indicate means ± SEM.

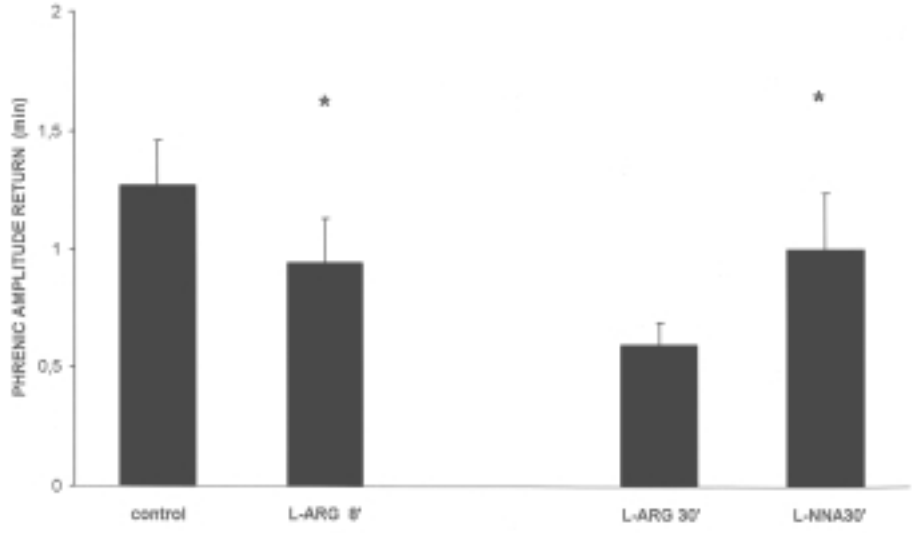

Fig. 5. Time of the decay of short--term potentiation of phrenic amplitude in the control and after L-Arginine and L-NNA administration. There is a significant difference (shown by asterisks) tested with Student's paired *t*-test between duration of the effect in control and after L-Arginine ($P<0.05$, $n = 11$) and between the effects observed 30 min. after L-Arginine and after L-NNA ($P<0.05$, $n = 8$). Bars indicate means ± SEM.

Table I

Effect of systemic administration of L-Arginine and L-NNA on mean arterial pressure, MAP, calculated from the record of the arterial blood pressure.

$n = 8$	Control	8' after L-ARG	30' after L-ARG	Control	8'after L-NNA	30'after L-NNA
MAP mmHg	78.06	72.61	73.84	71.00	71.66	76.41
SEM	4.94	6.59	4.51	3.76	4.12	4.46

30 seconds to 128.5% ± 9.9. This value was significantly lower than before and after L-Arginine. The duration of potentiation of hypoglossal activity was longer (2.2 ± 0.7 minutes) in comparison to the condition after L-Arginine pre-treatment and significantly shorter ($P<0.01$) in comparison to the control (Fig. 3).

L-NNA caused an insignificant increase in phrenic amplitude. Following SLN stimulation phrenic activity reached the control values immediately and a subsequent increase in amplitude was 106.7% ± 2.0 (Fig. 4); still, this small increase in amplitude lasted significantly longer than after L-Arginine (1.0 ± 0.3 minutes $P<0.05$) (Fig. 5).

Summarising, post-stimulatory effects of SLN stimulation on hypoglossal and phrenic activities were attenuated by L-Arginine. Effects of L-Arginine on respiration-related nerve activity during and after stimulation of SLN were only partially reversed by L-NNA.

DISCUSSION

The aim of this study was to test the hypothesis that nitric oxide modulates expression of short-term potentiation (STP) in hypoglossal nerve activity evoked by stimulation of the superior laryngeal nerve. This study demonstrates that alteration in NO availability by systemic application of either NO substrate or nitric oxide synthase (NOS) blocker can significantly change the short-term potentiation.

We use the expression "short-term potentiation" (Zucker 1989), introduced to respiration by Wagner and Eldridge (1991), although the phenomenon observed in our experiments has a somewhat shorter duration. It does, however, show several features of the short-term synaptic potentiation as for instance the exponential character of the decay.

Responses of hypoglossal and phrenic nerve activity during SLN stimulation

In the rabbit, responses of phrenic and hypoglossal activities to SLN stimulation were in general similar to those described for the cat (Jiang et al. 1991). However, SLN stimulation often evoked a depression of phrenic nerve activity and prolongation of the expiratory phase

rather than apnoea. Simultaneously, during the course of SLN stimulation hypoglossal activity revealed an increase in tonic activity with superimposed periodic bursts when phrenic discharged. This effect was attenuated slightly when stimulation was applied after NO substrate, L-Arginine, treatment and increased again when the inhibitor of NO synthesis, L-NNA, was given.

Knowledge about a possible role of NO, a biologically active molecule, in the central mechanisms controlling respiration is limited. It was shown in one study that endogenous NO formation is involved in mechanisms associated with respiratory rhythm generation (Ling et al. 1992). Lately, it was found in the brain stem preparation of the new-born rat (Volgin et al. 2000) that a perfusion of the preparation with NO substrate or NO synthase blocker evoked changes in frequency and amplitude of the respiratory burst. The direction of changes in the respiratory parameters depended on preserved continuity of neural structures. In addition, in amphibian, NO probably is involved in neuromodulation of the respiratory drive (Hedrick and Morales 1999).

The present study showed only weak modulation of the basal respiratory activity by changes in NO level. Fluctuations of the response during stimulation were not statistically significant, nevertheless, they may reflect a mechanism related to the nitric oxide availability during the transmission of information from SLN to hypoglossal motoneurones. The effects of NO substrate as well as NO synthase inhibitor on laryngeal stimulation in the current study might be associated with the function of glutamate receptors. There is an anatomical link between glutamatergic and nitroxidergic systems in the nucleus tractus solitarii and some physiological interactions between the two systems could occur through such a link (Lin et al. 2000). NMDA and non-NMDA glutamate receptors are present on the membranes of brain stem respiratory neurones (Pierrefische et al. 1991, Ling et al. 1992). NO is involved in cellular responses to stimulation of glutamate receptors especially of the NMDA subtype. Nevertheless, it is also involved in functioning of non-NMDA receptors because it causes a desensitisation of AMPA. Faster desensitisation exerts a dampening effect on action potentials. The SLN afferents terminate in several subdivisions of the nucleus of the solitary tract (NTS) where the enzyme synthesising NO is present and where NO plays a role in regulation of neuronal activity (Tagawa et al. 1994).

There is no available data on the role played by neurotransmitters in the transmission of excitation of the hypoglossal activity during SLN stimulation. It is known that SLN-evoked inspiratory termination of phrenic nerve activity involves most likely non-NMDA receptors localised within the region of the mNTS (Karius et al. 1993). Since NO facilitates the desensitisation of AMPA receptors, an increase in NO production evoked by L-Arginine may desensitise AMPA receptors more and in this way cause a lower excitation of hypoglossal activity during SLN stimulation.

Poststimulatory effects

The phenomenon of potentiation or augmentation of respiratory activity was described in the activity of phrenic (Budzińska et al. 1978, Eldridge and Millhorn 1986), hypoglossal, trigeminal and facial nerves (Mathew et al. 1982, St. John 1986), in response to the different inputs. Jiang and co-workers (1991) extensively studied the response of hypoglossal nerve to SLN stimulation in the cat. They suggested that prolonged augmentation of hypoglossal activity was achieved by increased synaptic input to hypoglossal motoneurones that lasts over the period of stimulation.

Nitric oxide plays an important role in neural plasticity. From studies on the mechanisms of CA1 hippocampal long-term potentiation, a model of synaptic plasticity that contributes to memory storage (Zorumski and Izumi 1993, Garthwaite and Boulton 1995), it is hypothesised that NO is essential for induction and maintenance of LTP. LTP is mimicked by NO-generating drugs and is inhibited by NOS inhibitors and haemoglobin (Mayer 1995). However, there is an increasing amount of information that NO synthase inhibition blocks the induction of long-term potentiation (Izumi et al. 1992, Zorumski and Izumi 1993, Garthwaite and Boulton 1995). Besides, postsynaptic activation of NMDA receptors has been implicated in neuronal potentiation, while activation of NMDA induces NO synthesis. For the respiratory neuronal complex it was found that postsynaptic activation of NMDA receptors mediates at least a portion of the respiratory neuronal potentiation observed following carotid sinus nerve stimulation (England et al. 1992). Short-term potentiation of the phrenic motor output evoked by paired-pulse stimulation is also mediated by NMDA receptors (McCrimmon et al. 1997).

In the present experiments, after systemic L-Arginine administration, short-term potentiation in hypoglossal activity induced by stimulation was diminished both in terms of amplitude and duration of the decay; however, the effect was not completely abolished by L-Arginine.

Such results do not support the idea of NO-induced respiratory potentiation. L-NNA, a competitive NOS inhibitor used in the present study, facilitates short-term potentiation. L-Arginine has the opposite effect. It is suggested that endogenous NO or related compounds could be physiological inhibitory factors used by neurones to modulate neuronal NMDA receptor activities (Kato and Zorumski 1993). Since NMDA activation induces NO synthesis it is also proposed that the administration of NO through a feedback mechanism results in inhibition of NMDA receptor activity (Manzoni et al. 1992, Ujihara et al. 1993). An analogous mechanism may underlie the inhibition of hypoglossal STP by NO precursor.

Poststimulatory effects observed in our studies might result from the action of NO on NMDA receptors of the respiratory neurones. Stimulation of endogenous nitric oxide synthase with L-Arginine caused increasing production of NO that can exert a feedback inhibitory effect on its sensitising enzyme and as a consequence attenuated NMDA-induced current. Application of the NO synthase inhibitor L-NNA decreased nitric oxide availability and partly reversed this effect. Another explanation of the diminished potentiation following L-Arginine is modulation of non-NMDA receptor activity by nitric oxide. As discussed above, NO desensitises AMPA receptors. This mechanism involves cGMP production (Garthwaite and Boulton 1995). It was found (Funk et al. 1995) that local application of cyclothiazide, a blocker of desensitisation of AMPA receptors, into the hypoglossal motor nucleus produced an increase of rhythmic synaptic drive in hypoglossal motoneurones.

A contribution to neural plasticity is implicated also for serotonin. Serotonin induced long-lasting modulation of the respiratory rhythmogenesis and phrenic and hypoglossal activity (Rose et al. 1995). Serotonin participates in such reflexes as cough or swallowing in which both laryngeal and hypoglossal activities take part. On the other hand serotonin does not influence the reflex response of the hypoglossal motoneurones to upper airway negative pressure (Douse and White 1996) although this reflex is transmitted at least partly by the superior laryngeal nerve (Mathew and Farber 1983). Thus, it seems that the serotonin pathway can be differentially involved in respiratory reflexes of laryngeal origin. Nevertheless, data on the influence of the NO pathway on divergent types of serotonin receptors in the central nervous system is, to our knowledge, still too modest to speculate on the mutual relationship of these two neurotransmitter pathways in the possible role in hypoglossal STP. In general it seems that serotonin is required rather for the long-term effects of the afferent stimulation than for the short ones. Correspondingly, in other systems than the respiratory one, serotonin takes part in long-term after-effects and is not engaged in the short-term effects (Bliss et al. 1983, Malyshev et al. 1997).

The nervous plasticity phenomena are also linked with brain-derived neurotrophic factor (BDNF) that is an important determinant of long-term potentiation, LTP (Korte et al. 1996). This neurotrophin probably takes part in serotonin dependent long-term plasticity in spinal respiratory motor control (Johnson et al. 2000). The BDNF increases the number of NADPH-diaphorase positive neurones (Huber et al.1995). It seems that both NO and BDNF interact in their production and activities (Xiong et al. 1999). In the NTS, a structure important for respiration where vagal afferents terminate, BNDF inhibits AMPA-mediated current. It is suggested that BDNF may regulate glutaminergic transmission at primary afferent synapses (Balkowiec et al. 2000). The question whether BDNF is also involved in short-term respiratory after-effects of laryngeal afferent stimulation in association with NO and AMPA is worthy of study. Besides the glutaminergic, serotoninergic and neutrophin systems mentioned above there is a variety of neurotransmitters and neuromodulators influencing processes related to neural plasticity, mostly long-term, that may take part in respiratory potentiation.

The present study cannot discern the exact mechanism of NO contribution to respiratory short-term potentiation but we could find that the phenomenon is modulated by systemic invasion into NO production. We chose the systemic way of administration of the NO substrate and NOS inhibitor being aware of the limitations in the interpretation of the results. In several brain stem structures related to the respiratory control system, one can observe signs of shorter or longer transient potentiation or depression present beyond the period of afferent stimulation. However, it is not known exactly in which part of the central nervous system the processing of the information causing short--term potentiation of respiratory activity takes place. Thus, the systemic way of penetration into the NO system seemed to be the best one to learn whether or not nitric oxide participates in the STP.

In the case of systemic application of NO active substances, just as in the case of other routs of administration such as intracerebroventricular injections or microinjections into specific neural structures, one cannot also

avoid certain cardiovascular effects (Tseng et al. 1996) and respiratory effects (e.g. Ling et al. 1992). Cardiovascular responses have implication for the central control of breathing. We found that in the rabbit the L-Arginine effect on blood pressure was evident only during the first few minutes, subsiding within 6-8 minutes from the injection. L-NNA chosen for present experiments evokes a small increase in blood pressure and, in contrast to other NOS-blockers, has a weaker effect on the baroreflex (Wojtal et al. 1995, Liu et al. 1996). 30 mg/kg of L-NNA used in present experiments on rabbits caused a smaller increase in blood pressure than reported for 40 mg/kg in the cat (Teppema et al. 1997). The difference in the magnitude of the response may depend not only on the dose of substance but also on the sequence of the substance applications; L-Arginine was given first and L-NNA second in our experiments. It is worth mentioning that isoprenaline, having a similar net effect on arterial blood pressure as L-Arginine, exerts an opposite effect on hypoglossal short-term potentiation - it enhances STP (Wojtal and Budzińska 1996). Although L-Arginine influences blood pressure, the responses of hypoglossal and phrenic activities to SLN stimulation were studied when the blood pressure was stabilised. Thus, respiratory responses to SLN stimulation cannot be attributed to changes in blood pressure.

Different from the findings of Jiang and co-workers (1991), our study on rabbits showed that it was possible also to see in some of the experiments weak augmentation of phrenic nerve activity decaying after removal of SLN stimulation much earlier than that of hypoglossal. This effect might indicate that the SLN input, in spite of diverse effects on phrenic and hypoglossal activity during stimulation, excites a common group of neurones or network involved in mechanisms of the respiratory activity potentiation.

In conclusion, changes in NO production evoked by either an increase of NO substrate or an inhibition of NO synthesis influence the poststimulatory effects in hypoglossal nerve activity resulting from SLN stimulation. NO attenuates short-term potentiation of the hypoglossal activity. The attenuation only, but not abolition of the response by L-Arginine, indicates that potentiation can be produced by more than one mechanism that involves nitric oxide. Nitric oxide might be a negative modulator of the transmission of short-term potentiation in hypoglossal and phrenic activity. Interactions between NO-mediated events and those mediated by other receptors or neuromodulators systems still need explanation. Further studies on mechanisms of the respiratory potentiation are necessary.

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