ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LVII, N 1, 10 SECTIO D 2002

Chair and Department of Human Physiology, Medical University of Lublin

STEFAN DYBA, INGEBORGA TYCHOWSKA,
LIDIA KLUKOWSKA, ANNA NADULSKA

The influence of baclofen on reflex circulatory reactions evoked by stimulation of the vagus nerve in the rabbit

GABA-mechanisms play a significant role in regulation of circulatory parameters such as: arterial blood pressure and heart rate. They also have a regulatory influence on the respiratory action of animals. Nucleus of the solitary tract (NTS) is the place where cardiovascular afferent fibres from arterial baroreceptors, chemoreceptors and receptors from cardiopulmonary area converge (6, 7). Therefore this place plays an important role in directing the autonomic control of the cardiac muscle and vessels as a response to releasing the reflexes from the afferents of the vagus nerve – i.e. glossopharyngeal, among other things. Numerous studies have indicated that a stimulating neurotransmitter in NTS, which is involved in the cardiovascular reactions, is amino acid L-glutamate (5). In NTS also other neurotransmitters involved in the transmission of information from chemo – and baroreceptors have been confirmed. There are particularly numerous GABA-ergic neurons and $GABA_A$ and $GABA_B$ receptors (1, 4). Local injection of GABA as well as of the agonists of $GABA_A$ and $GABA_B$ receptors evoked the increase in mean arterial blood pressure and cardiac rate, which seems to be the result of the blockade of the sympathoinhibitory component of baroreflex (3). Microinjection of muscimol, a $GABA_A$ agonist, into NTS also reduced the cardiovagal component of the baroreflex as well as hypotensive and bradycardiac responses to electrical stimulation of the aortic and vagal depressor nerve. Studies with local administering of GABA agonists were carried out by injecting baclofen into a hypothalamic pressor area (ventro-medial hypothalamus) and into a depressor area (anterior hypothalamus). Baclofen injected into ventromedial hypothalamus decreased sympathetic nerve activity, blood pressure and the heart rate. The same doses of baclofen injected into the anterior hypothalamus increased blood pressure and the heart rate (9). Baclofen injected intravenously causes dose-dependent circulatory reactions in rats. Small doses cause the decrease in pressure and heart rate; bigger doses result in the increase in pressure, acceleration of the heart rate and ectasis

of cutaneous vessels. This study aims at testing the effect of baclofen injected intravenously on the values of circulatory parameters such as mean arterial blood pressure and the heart rate and their change influenced by stimulation of centripetal sections of vagal nerves in a rabbit.

MATERIAL AND METHOD

The experiments were carried out on 30 rabbits of both sexes, crossbreeds, with body weight 3-5 kg. The 20% solution of urethane at a dose of 1.5 g/kg was used for anaesthesia. The skin and subcutaneous tissue was cut along the central body line from the lower jaw bone to the upper edge of sternum. After uncovering of the trachea, it was cut across along the lower edge of the thyroid gland and the tracheotomic tube was inserted. Vagus nerves were prepared on both sides. In order to record the arterial pressure a tube filled with Ringer fluid with heparine was inserted into the ear middle artery lumen and it was connected with the electromagnetic converter of electric manometer. Mean arterial blood pressure and cardiac action were registered on the magnetic carrier of a computer. For stimulation of central sections of vagus nerves platinum electrodes were used and they were connected to a square wave oscillator. To prevent the nerves from drying off, they were submerged in warm paraffin oil. The temperatures of oil and the animal were maintained at 37±0.5 °C. When the initial actions had been finished the animals were administered 2,500 units of heparine. The actual experiment was initiated after an hour. The intensity of stimulation was expressed by multiple threshold stimulation (T) and it was the repeated reaction of pressure drop equal to 1–4 mm Hg. Square wave pulses with the following parameters were used for stimulation: frequency – 5 cycles per second, time – 20 seconds, the width of individual impulse – 1 msec. The intervals between stimulation were equal to 5 minutes. Baclofen at the dose of 1.0 mg/kg administered to the auricular vein was used for stimulation of $GABA_B$ receptors.

RESULTS

During the first series of experiments the value of pressure drop reaction and heart rate change during stimulation of centripetal sections of the right and then left vagal nerve and then both of them simultaneously with stimuli of increasing value from 1 to 5 T, were tested. The sum of unilateral effects was compared with the value of effects obtained during simultaneous bilateral stimulation of vagal nerves. When the sum was lower than the effect of bilateral simultaneous stimulation, it indicated facilitation of the circulatory reaction value; when it was higher – it suggested its occlusion. During the second series of experiments this test was repeated after injecting of baclofen and waiting

for 40 minutes until destabilisation of circulatory parameters connected with administering of the preparation has completely receded. Mean arterial blood pressure was 117.2 mm Hg, the heart rate was 292.7 beats per minute. Injecting of baclofen caused the increase in the heart rate by 16±5 beats per minute on the average. Mean blood pressure was also increased by 15±5 mm Hg on the average. The results of the changes are presented in Figure 1 and Figure 2.

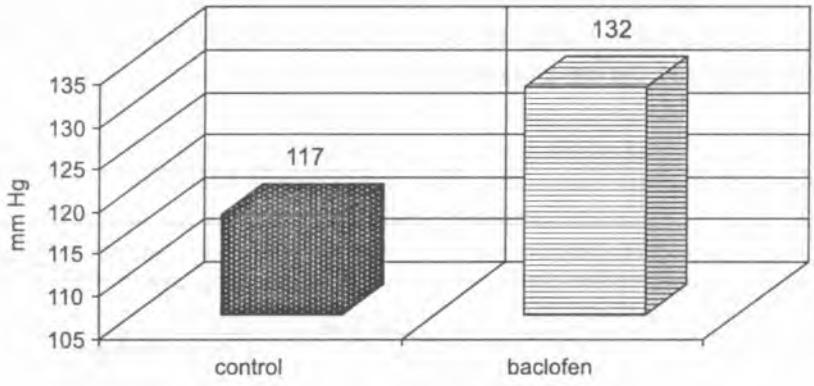

Fig. 1. Effect of baclofen on initial arterial blood pressure

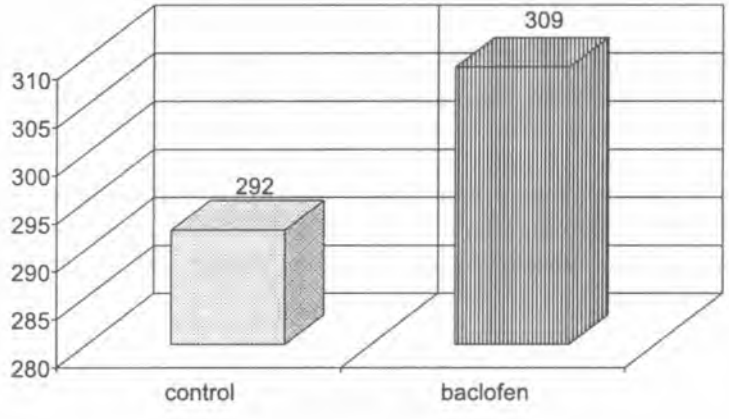

Fig. 2. Effect of baclofen on the initial heart rate

In the control group facilitation of pressure reaction and of the heart rate was observed only with the lowest intensity of stimulation 1–1.5 T. Then the sum of the unilateral effects was bigger than the effect of simultaneous bilateral stimulation. When higher parameters of the intensity of stimulus were used, occlusion characterised by the domination of the sum of unilateral effects over the effect of simultaneous bilateral stimulation was observed. This occlusion was intensified with the increase in the intensity of stimulus up to the value of 4-5 T. With higher values of the stimulus intensity it minimally progressed (2). After injection of baclofen we did not observe facilitation of circulatory reactions with any of the applied values of stimulation force. Only occlusion was present and it was intensified as in the control group up to 4–5T. (Fig. 3).

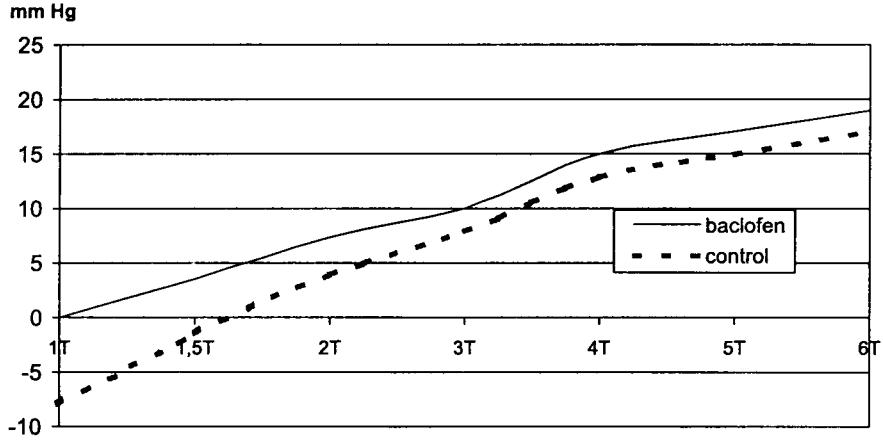

Fig. 3. Correlation between the size of the area of active overlapping of the afferent innervation of the vagus nerves and the strength of the stimulus

Injection of baclofen caused simultaneous decrease in pressure drop reaction and slowing down of the heart rate with the same, as in the control group, intensity of stimulation for arterial blood pressure by 16±7% on the average, and for the heart rate by 20±9 beats per minute.

DISCUSSION

Numerous studies having been carried out so far prove that stimulation both of $GABA_A$ and $GABA_B$ receptors in nucleus tractus solitari caused inhibiting of the reflex value of circulation and heart response with stimulation of afferents spreading out from the arterial baroreceptors (7, 8). During electrophysiologic tests attention was drawn to

the fact that stimulation of $GABA_A$ receptors caused a generalised reaction of decreasing the discharge of barosensitive neurones not correlated with the heart rate, however the stimulation of $GABA_B$ receptors with baclofen inhibited the discharge of neurones synchronically with pulse. The observed increase of mean arterial blood pressure and of the heart rate after injection of baclofen is caused probably by the decrease of neuronal activity, which inhibits the activity of sympathetic neurones involved in the responses from the arterial baroreceptors. Possibly the effect is caused by stimulation of presynaptic $GABA_B$ receptors in synaptic connectios in nucleus tractus solitari with the afferents of arterial baroreceptors. Stimulation of these receptors inhibits the release of amino acid glutamate transmitter and this results in the removal of the tonic sympathicoinhibitory activity of the baroreflex afferents. In the stimulated stems of vagus nerves there are also fibres spreading from arterial chemoreceptors. As many studies point out the reaction of the increase of mean blood arterial pressure that takes place during their stimulation is also connected with the activation of $GABA_B$ receptors.

The disappearance of facilitation of heart and pressure reactions after injection of baclofen seems to be an interesting fact. It shows that after stimulation of $GABA_B$ receptors there is an increase in overlapping of the afferent innervation from both vagal nerves in a widely comprehended cardiovascular centre.

CONCLUSIONS

Baclofen, being a selective agonist of $GABA_B$ caused:

1. The increase in mean blood pressure and heart rate.
2. Weakening of the vascular and cardiac reflex reaction component caused by stimulation of vagal afferents.
3. The increase of mutual overlapping of afferents from both vagal nerves and this was manifested by the elimination of facilitation with low intensity of stimulation.

REFERENCES

1. Bowery N. et al.: $GABA_A$ and $GABA_B$ receptor binding site distribution in the rat central nervous system. Neuroscience, 20, 365, 1987.
2. Dyba S.: Facilitation, occlusion and conditioning of pressure responses evoked by developing during aortic nerve stimulation in rabbit. Acta Physiol. Pol., 103, 1981.
3. Florentino A. et al.: Mechanism of the cardiovascular effects of $GABA_B$ receptor activation in the *nucleus tractus solitarii* of the rat. Brain Res., 535, 264, 1990.

4. Gale K. et al.: The presence of GABA and specific GABA binding sites in cat brain nucleus associated with central vagal outflow. Brain Res. Bull., 5, Suppl. 2, 324, 1980.
5. Okeda M. et al.: Contrasting baroreflex effects of muscimol versus bicuculine injected into the *tractus solitarius* in anesthetized rats. J. Cardiovasc. Pharmacol., 26, 363, 1995.
6. Palkovits M, Zaborszky L.: Neuroanatomy of central cardiovascular control. *Nucleus tractus solitarii*: afferent and efferent neuronal connections in relation to the baroreceptor reflex arch. Prog. Brain Res., 47, 9, 1977.
7. Sevoz C. et al.: Role of serotonin$_3$ receptors in the *nucleus tractus solitarii* on the carotid chemoreflex. Am. J. Physiol., 272, 1250, 1997.
8. Sved A. F., Gordon F. J.: Amino acids as central neurotransmitters in the baroreceptor reflex pathway. News Physiol. Sci., 9, 243, 1993.
9. Takenaka K. et al.: Hypothalamic and medullary $GABA_A$ and $GABA_B$ ergic systems differently regulate sympathetic and cardiovascular systems. Clin. Exp. Pharmacol. Physiol., 22, Suppl. 1, 48, 1995.

2001.05.02

SUMMARY

The purpose of our investigations was to investigate the effect of baclofen upon the reflex fall of arterial blood pressure and heart rate evoked by stimulation of bilateral vagus nerves. The experiments were carried out on 30 rabbits of both sexes and mixed breed weighing 3000-4500g under urethane anaesthesia (1.5 g/kg). Mean arterial pressure and heart rate were measured in the ear middle artery by the electromanometer and registered by PC computer. Parameters of stimulation were: frequency 5c/s., duration – 20 s., width of single rectangular pulse – 1 msec., intensity of stimulation – multiplicity of threshold excitation (T). The intervals between stimulations were not shorter than 5 min. Baclofen was administrated intravenously (1.0 mg/kg). In the control group the facilitation of circulatory reaction was observed only at the smallest intensity of stimulation (1.0T–1.5T). When greater intensity of stimulations was applied the sum of unilateral effects was greater than the effect of simultaneous bilateral vagus nerve stimulation. This occlusion effect increased up to the stimulus value of 4-5T. After baclofen administration the facilitation of circulatory responses disappeared and only occlusion was observed. Baclofen increased the size of the area of active overlapping of the afferent innervation of the vagus nerve. Administration of baclofen produced an increase in mean arterial blood pressure (15 ±6 mm Hg) and heart rate (16±5 beats per minute) and decrease in the vagal reflex fall of mean arterial pressure and heart rate. Stimulating of the $GABA_B$ receptors markedly attenuates the baroreceptor reflex resulting in increase in mean arte-

rial blood pressure and heart rate. These results support the hypothesis that GABA acts tonically on $GABA_B$ receptors to attenuate the baroreceptor reflex, thereby contributing to the regulation of circulatory parameters.

Wpływ baklofenu na reakcje krążeniowe wywołane drażnieniem nerwu błędnego u królika

Celem naszych doświadczeń było zbadanie wpływu baklofenu na odruchowe zmiany ciśnienia tętniczego krwi i akcji serca, wywołane obustronnym drażnieniem nerwów błędnych. Doświadczenia przeprowadzono w narkozie uretanowej (1,5g/kg) na 30 królikach mieszańcach obu płci o wadze 3000–4500. Średnie ciśnienie krwi i częstość akcji serca rejestrowano w tętnicy środkowej ucha przy użyciu czujnika elektromagnetycznego i rejestrowane ono było na nośniku magnetycznym komputera. Drażniono dośrodkowe odcinki prawego, lewego i jednocześnie obu nerwów błędnych. Stosowane parametry drażnienia były następujące: częstotliwość 5c/sek., czas drażnienia - 20 sek., szerokość pojedynczego impulsu 1 msek. Siłę drażnienia wyrażano wielokrotnością pobudzenia progowego (1 T), za które uważano powtarzalny spadek ciśnienia wynoszący 2-4 mm Hg. Odstępy pomiędzy drażnieniami wynosiły 5 min. Baklofen podawano dożylnie w dawce 1,0 mg/kg masy ciała. W grupie kontrolnej torowanie reakcji krążeniowych obserwowano jedynie przy najmniejszych intensywnościach drażnienia (1,0 – 1,5T). Przy silniejszych drażnieniach suma efektów jednostronnych była większa od wielkości uzyskiwanych podczas jednoczesnego obustronnego drażnienia nerwów błędnych. Ten efekt okluzji narastał w miarę zwiększania siły bodźca do wielkości 4–5T. Po podaniu baklofenu zjawisko torowania przy najmniejszych intensywnościach siły bodźca znikało. Obserwowano jedynie okluzję. Baklofen podnosił średnie ciśnienie krwi tętniczej o 15±6 mm Hg i częstość akcji serca o 16±5 uderzeń na minutę. Jednocześnie obserwowano zmniejszenie wielkości odpowiedzi odruchowych przy tych samych wartościach siły drażnienia. Powyższe wyniki wspierają hipotezę, że GABA poprzez receptory $GABA_B$ osłabiają wielkość odpowiedzi odruchowej z aferentów błędnych i w ten sposób przyczyniają się do regulacji parametrów krążeniowych.