ROLE OF GABA AND GLYCINE IN THE FORMATION OF SOMATO-SYMPATHETIC REFLEXES

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The action of strychnine and picrotoxin, synaptic antagonists of glycine and GABA, on the components of somato-sympathetic reflexes evoked by stimulation of cutaneous and intercostal muscular afferents was studied in experiments on intact or cordotomized cats. Evoked potentials were recorded in the white ramus communicans of the 3rd thoracic segment. Strychnine and picrotoxin produced different changes in the magnitude of the various components of the reflex, indicating differences in the localization of the inhibitory action of GABA and glycine in the central link of the somato-sympathetic reflex arc.

KEY WORDS: *GABA; glycine; somato-sympathetic reflexes.*

According to recent findings [11, 13], GABA and glycine occur in large quantities in the spinal cord, where they perform the role of inhibitory mediators in segmental neuronal systems. Many nerve cells of different types (interneurons, motoneurons, sacral parasympathetic preganglionic neurons) are sensitive to the action of these neuroactive amino acids [9, 14]. GABA and, perhaps, glycine perform the function of mediators of presynaptic inhibition of spinal reflexes [6, 8].

The question of the role of these amino acids in somato-sympathetic reflexes at the spinal level has so far received little study. The writers previously studied the effect of GABA and its phenyl analog β-phenyl-γ-aminobutyric acid on the various components of somato- and viscero-sympathetic responses recorded in postganglionic sympathetic fibers [1]. The further study of the action of inhibitory amino acids on segmental mechanisms of regulation of autonomic functions (including the function of maintenance of vascular tone) is of considerable interest because increasingly great importance is being attached to spinal neurons in the mechanism of somato- and viscero-sympathetic reflexes. In the opinion of several investigators [5, 7] all components of somato-sympathetic reflexes can be reproduced at the segmental level.

The object of this investigation was to study the role of GABA and glycine in the processes of formation of somato-sympathetic reflexes. A convenient experimental method for achieving this goal is to record evoked potentials from axons of sympathetic preganglionic neurons (SPN) running in the white ramus communicans (WRC) in response to stimulation of spinal afferents. Under these circumstances the action of the test amino acids at the ganglionic level is excluded. For pharmacological analysis of the role of GABA and glycine in the mechanism of somato-sympathetic reflexes substances blocking the synaptic action of glycine (strychnine [10]) and GABA (picrotoxin [12]) were used.

EXPERIMENTAL METHOD

Experiments were carried out on 17 cats anesthetized with chloralose and pentobarbital (45 and 10 mg/kg, respectively) by intraperitoneal injection. Evoked potentials were recorded in WRC of the 3rd thoracic segment in response to stimulation of cutaneous, muscular, and mixed branches of the segmental intercostal nerve of the same segment by single supra-

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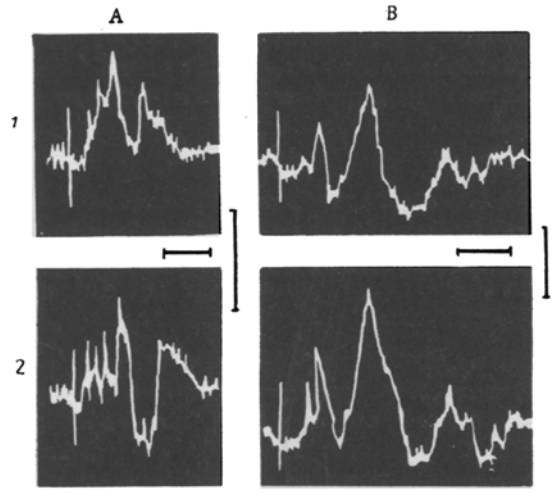

Fig. 1

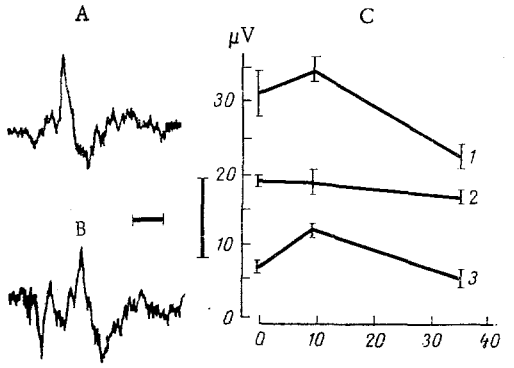

Fig. 2

Fig. 1. Effect of picrotoxin on characteristics of early response to stimulation of cutaneous afferents (A) and of mixed branch of segmental nerve (B): 1) before injection of picrotoxin; 2) 10 min after intravenous injection of 0.2 mg/kg picrotoxin. Calibration: time, for A and B, 10 msec; amplitude, for A, 10 μV, for B, 20 μV.

Fig. 2. Effect of strychnine on components of early response to stimulation of mixed branch of intercostal nerve: A) before, B) 15 min after injection of strychnine in a dose of 0.5 mg/kg. Calibration: time 5 msec, amplitude 20 μV. Beginning of sweep of beam corresponds to stimulus marker. C) Change in amplitude of components of early response at different time intervals after injection of 0.5 mg/kg strychnine: 1) ER-2, 2) ER-3, 3) ER-1. Mean results of five experiments.

TABLE 1. Effect of Strychnine and Picrotoxin (0.2-0.5 mg/kg) on Characteristics of Early Response (ER) to Stimulation of a Mixed Branch of Intercostal Nerve

Components of ER	Animals	Initial data			7-20 min after injection of strychnine
		latent period, msec	amplitude, μV	duration, msec	latent period, msec
ER-1	With intact spinal cord	5,41±1,83	9,64±0,84	2,34±0,87	6,0±1,6
	Spinal	5,12±1,6	9,61±2,9	2,37±0,81	5,52±1,5
ER-2	With intact spinal cord	13,1±1,33	32,7±1,02	12,71±1,85	12,26±2,29
	Spinal	12,5±2,6	25,44±7,4	12,3±3,2	12,18±2,8
ER-3	With intact spinal cord	24,9±6,01	18,02±0,21	10,8±5,01	24,7±2,61
	Spinal	22,03±4,35	16,94±5,8	8,93±2,04	21,3±5,03

Components of ER	Animals	7-20 min after injection of strychnine		7-20 min after injection of picrotoxin		
		amplitude, μV	duration, msec	latent period, msec	amplitude, μV	duration, msec
ER-1	With intact spinal cord	13,83±0,6*	2,0±0,24	5,6±0,87	15,24±4,5*	3,11±1,5
	Spinal	13,79±1,9	3,6±1,7	5,25±0,98	15,35±0,7*	3,41±1,1
ER-2	With intact spinal cord	39,7±3,2	13,4±1,65	13,08±0,55	46,52±1,7*	12,72±2,03
	Spinal	23,36±7,6	11,9±3,14	11,0±1,0	44,33±2,07*	16,96±1,72
ER-3	With intact spinal cord	16,9±4,5	10,3±3,8	22,2±4,5	38,71±2,26*	14,06±4,3
	Spinal	13,44±3,39	7,92±1,67	20,24±0,5	39,35±2,4*	12,1±7,7

Legend. Statistically significant differences marked by asterisk.

maximal electrical pulses (0.1-0.2 msec, 6-10 V). The spinal cord was divided at the level of the 5th cervical segment by means of an ultrasonic knife. The blood pressure was recorded in the common carotid artery by the usual method. The animals were immobilized with myorelaxin (suxamethonium) and artificially ventilated. The strychnine and picrotoxin used for pharmacological analysis were injected intravenously in doses of between 0.2 and 0.5 mg/kg body weight.

EXPERIMENTAL RESULTS AND DISCUSSION

In cats with an intact spinal cord stimulation of afferent fibers of a mixed branch of the segmental nerve evoked two types of responses in succession: an early (ER) response with a latent period of its first component of 4.5 to 6.2 msec, and a late (LR) response with a latent period of 35-42 msec. The ER is known to be heterogeneous in structure and to consist of three principal components: ER-1, ER-2, and ER-3 [3].

The first component (ER-1), with the shortest latent period, was probably monosynaptic in nature. SPN participating in the formation of the last two components (ER-2 and ER-3) were activated polysynaptically [3, 4]. In the present experiments in response to stimulation of a mixed branch of the intercostal nerve an ER differentiated into three components (Fig. 1B), the electrophysiological characteristics of which are given in Table 1, also was observed.

Since the mixed branch of the segmental nerve contains cutaneous and muscular afferent fibers as well as some fibers from the pleura [15], it was natural to postulate a connection between the appearance of the individual components of ER and activation of different groups of afferents. The experiments showed that stimulation of cutaneous fibers led to the appearance of a monosynaptic ER-1 and of the main response with a latent period of 9-11.5 msec. Stimulation of the muscular branch of the segmental nerve led to the appearance of a wave in WRC with a long latent period (14.5-18 msec).

ER-1 recorded from WRC consisted of the sum of the action potentials of a small number of SPN, which often did not merge into a single wave but were recorded as separate spikes (Fig. 2B). ER-1 is known to be formed by discharges of SPN located in the anterior horns of the spinal cord (B_1-SPN) [3], with fast-conducting (up to 20 m/sec) axons. Strychnine and picrotoxin, in doses of 0.2-0.5 mg/kg led either to an increase in the amplitude of the synchronized wave of ER-1 by 40-50% or (more often) to the appearance of additional B_1-SPN spikes within the time of appearance of ER-1 in both the intact and the spinal animals (Fig. 1A, 1 and 2). These results could indicate the presence of GABA- or glycinergic inhibition of activity of some B_1-SPN, which was abolished by the convulsants used.

Picrotoxin, in doses of 0.2-0.3 mg/kg, increased the amplitude of the polysynaptic components of ER in cats with an intact spinal cord and in the spinal animals: ER-2 by 50% and ER-3 by 70% (Fig. 1B, 1 and 2). Strychnine, when injected systemically in doses of 0.2-0.5 mg/kg, caused no significant change in the characteristics of ER-3 but slightly increased the amplitude of ER-2 (Fig. 2A and B). No facilitatory action of strychnine on ER-2 was observed after division of the spinal cord.

Strychnine and picrotoxin, in doses of 0.2-0.5 mg/kg, led to a marked increase in amplitude of the main component of ER evoked by activation of cutaneous afferents in both intact and spinal cats. Picrotoxin in doses of 0.3-0.5 mg/kg increased by 53% the amplitude of ER to stimulation of the muscular branch of the segmental nerve in cats with connections between the spinal cord and the brain intact. Even in a dose of 1 mg/kg, strychnine did not change the ER to excitation of muscular afferent fibers.

If ER represents the mass of reflex discharges of SPN of one or several segments, LR reflects the generalized response of SPN of many segments of the spinal cord to afferent activation [5]. In the present experiments an LR was regularly obtained in cats with an intact spinal cord and in four experiments on animals with a divided spinal cord. LR with a latent period of 35-42 msec and of relatively low amplitude (about 15 μV) and between 12.5 and 20 msec in duration appeared in response to stimulation of cutaneous, muscular, and mixed branches of the segmental nerve. Strychnine and picrotoxin, in doses of 0.2-0.5 mg/kg, more than doubled the amplitude of LR in the spinal animals and also in the cats with an intact spinal cord.

These results point to a role of GABA and glycine in the formation of different components of the somato-sympathetic reflexes at the segmental and suprasegmental levels. The point of application for the action of the two convulsants used could be GABA- and glycinergic synapses participating in the mechanism of inhibition of primary afferents, interneurons, SPN directly, and also the neuronal systems of the hypothetical age inhibition of SPN [2]. These observations do not answer the question of the concrete localization of the action of the GABA and glycine antagonists, but there is no doubt that these neuroactive amino acids have different types of influence on the activity of neuronal systems forming the different components of somato-sympathetic reflexes at the spinal and suprasegmental levels.

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ANALYSIS OF THE EFFECT OF MORPHINE AND TRIMEPERIDINE ON THE UPTAKE AND LIBERATION OF NORADRENALIN BY MYOCARDIAL TISSUE

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The effect of morphine and trimeperidine on the concentration, uptake, and liberation of noradrenalin (NA) in the rat myocardium was investigated. Trimeperidine lowers the NA level in the myocardium. Morphine does not affect the liberation of NA-^{14}C from the isolated perfused heart, whereas trimeperidine significantly increases it, affecting both the "slow" and the "rapid" release of the mediator. Trimeperidine does not affect the uptake of NA-^{14}C by the perfused heart but morphine significantly lowers it. Competition between morphine and NA is characterized by an incomplete inhibition effect: Morphine and NA mutually affect the affinity of each other for the receptor and their interaction depends on their relative concentrations.

KEY WORDS: *Noradrenalin; morphine; trimeperidine; myocardium.*

Morphine and trimeperidine are frequently used for the relief of the pain syndrome in ischemic heart disease and myocardial infarction. The central depriming effect of analgesics on tonic and reflex activity in the sympathetic nerves of the heart is connected with an increased concentration of functionally active forms of noradrenalin (NA) in the brain tissue [2]. The writer showed previously [3] that morphine lowers the NA level in the myocardium of rats and increases the concentration of free NA in perfusion fluid from the isolated rabbit heart. However, the question of whether trimeperidine influences NA metabolism

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