ANALGESIC EFFECTS OF INHIBITORY MEDIATORS IN A PAIN SYNDROME OF SPINAL ORIGIN

E. I. Danilova, V. N. Grafova, and G. N. Kryzhanovskii*

UDC 612.884-06:612.822.2

A pain syndrome of spinal origin was induced by creating a generator of pathologically enhanced excitation (GPEE) in the dorsal horn of the lumbosacral segments of the spinal cord in rats with the aid of tetanus toxin, strychnine, penicillin, KCl, and ouabain. The substances were applied to the dorsal surface of the spinal cord. An "agar wafer" method was developed to enable local prolonged and dose-dependent effects of the substances applied to be produced and studied in freely behaving animals. The effects of the inhibitory mediators were determined by the nature of the generator produced and, in particular, by the state of the mediator-receptor system of the neuronal membranes of cells forming in GPEE. Glycine prevented the development of the pain syndrome in the case of generators induced with tetanus toxin, penicillin, KCl, and ouabain. GABA was effective in the case of generators produced by tetanus toxin, potassium, and ouabain. Injection of glycine into the region of a generator produced by tetanus toxin at the height of development of the pain syndrome abolished that syndrome for the duration of action of the mediator. The pain syndrome could thus be specifically abolished or prevented for the period of action of the mediator by depressing the GPEE with the appropriate inhibitory mediators, depending on the nature of the GPEE, i.e., on the features distinguishing the state of the mediator apparatus of its neurons. Under these conditions inhibitory mediators, although not analgesics in the pharmacological sense, have a specific analgesic effect.

KEY WORDS: pain syndrome of spinal origin; determinant structure; generator of pathologically enhanced excitation; analgesia; inhibitory mediators.

The formation of generators of pathologically enhanced excitation (GPEE) [3, 7], which lie at the basis of hyperactive determinant structures, in certain parts of the CNS leads to the appearance of corresponding neuropathological syndromes [3]. By creating a GPEE in the nociceptive system, pain syndromes of central origin have been produced: spinal [1, 2, 4], trigeminal [2, 6], and thalamic [2, 5]. Since the origin and activity of the GPEE are connected to some degree or other with a disturbance of inhibitory processes [3, 7], it was considered important to study the effects of inhibitory mediators in these pain syndromes.

The object of the present investigation was to study the effects of glycine and γ-aminobutyric acid (GABA) in a pain syndrome of spinal origin induced by a GPEE in the dorsal horns of the spinal cord. The GPEE was produced by means of various substances, so that the resulting GPEEs differed in their pathogenetic structure.

EXPERIMENTAL METHOD

Albino rats weighing 200-250 g were used. A GPEE was induced either by direct disturbance of inhibitory processes in the system of dorsal horn nuclei with the aid of substances (tetanus toxin, strychnine, penicillin) disturbing different types of inhibition [8, 10, 12, 13], or indirectly with the aid of substances causing depolarization of neurons (potassium ions and ouabain) [14]. An "agar wafer" method was evolved to produce a long-acting effect of these drugs. The substance for use was added to a warm solution of 1% agar, carefully

* Corresponding Member of the Academy of Medical Sciences of the USSR.

Laboratory of General Pathology of the Nervous System, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 6, pp. 525-528, June, 1979. Original article submitted July 10, 1978.

0007-4888/79/8706-0545$07.50

TABLE 1. Effects of Glycine and GABA in Pain Syndromes Induced by GPEEs of Different Nature

Substance used to produce GPEE	Mediators	Number of animals	Latent period of onset of pain syndrome (from time of application of substance), min	Duration of pain syndrome, h	Degree of depression of pain syndrome during action of mediator
Tetanus toxin	Glycine	6	200±40	17±5*	+++
	GABA	5	120±30	18±5*	+++
	Control	5	40±10	20±5*	—
Strychnine	Glycine	6	10±5	2±0,5	—
	GABA	5	40±10	1,5±0,2	+—
	Control	5	10±5	2±0,5	—
Penicillin	Glycine	6	Pain syndrome did not develop	—	+++
	GABA	6	10±5	1,5±0,5	—+
	Control	5	10±5	3±0,5	—
KCl	Glycine	5	Pain syndrome did not develop		+++
	GABA	5	" " " "		+++
	Control	5	40±10	1,5±0,5	—
Ouabain	Glycine	5	Pain syndrome did not develop		+++
	GABA	5	" " " "		+++
	Control	5	40±10	1,5±0,5	—

*When tetanus toxin was applied the pain syndrome continued until death of the animal.

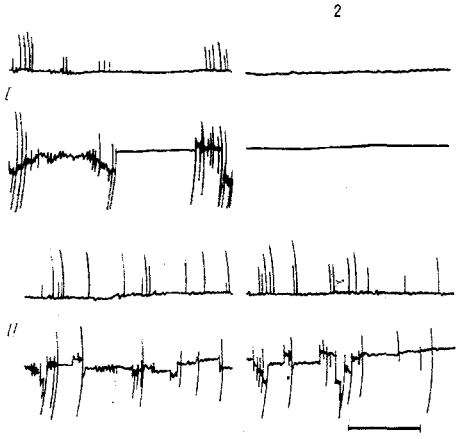

Fig. 1. Effect of glycine injected into posterior horns of lumbar segments of spinal cord on pain syndrome of spinal origin. In both parts (I and II) of figure top curves are phonograms and bottom curves actograms recorded at height of development of pain syndrome of spinal origin in a rat before (1) and 20 min after (2) microinjection of 20% glycine solution (I) and 8% NaCl solution (control, II), in a dose of $1 \cdot 10^{-4}$ ml for each injection, into posterior horns of segments L2-L6 on the left side. Tape winding speed (horizontal line) 3 cm in 5 min.

mixed, and when the agar had solidified, a wafer measuring 10 × 4 × 1.5 mm was cut out and applied to the dorsal surface of segments L2-L6 of the spinal cord on one side. To minimize trauma the spinal cord was exposed only unilaterally. The following substances were added to 1 ml of 1% agar: 1 ml tetanus toxin containing 15-50 MLD for rats, 1 ml 0.1% strychnine, 1 ml penicillin containing 15,000 units, 1 ml 75 mM KCl, 1 ml 0.5 mM ouabain, 1 ml 20% glycine, and 1 ml 1% GABA. Each of these mediators was deposited in the agar wafer together with the agent inducing GPEE formation. In one series of experiments glycine was injected into the dorsal horns of the same segments, by means of a microinjector (20% solution, in doses of $1 \cdot 10^{-4}$ ml; in control experiments 8% NaCl solution, in a dose equal to an iso-osmotic solution of glycine with the same pH, was injected), at the height of development of the syndrome. To allow for the possible mechanical effect of the agar wafer on the spinal cord, it was applied under the same conditions but without the substances. The "agar wafer" method, as described above, enables substances to be applied to the dorsal surface in different

doses, the period of their action to be lengthened, and the development of the syndrome to be observed in freely behaving animals. Still and motion picture photographs were taken, and in some experiments an actogram and phonogram were recorded.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. They show that glycine or GABA, if applied together with tetanus toxin, prevented development of a pain syndrome during the action of the mediator. Once it had developed, the pain syndrome then quickly increased and its intensity became the same as that in the control animals. A series of experiments showed that microinjection of glycine at the height of development of the syndrome induced by a "tetanus" generator completely suppressed the pain syndrome: The animals became quiet, the attacks of pain disappeared, and application of the stimulus to the trigger zones did not evoke a nociceptive response. The clinical picture described above occurred only during the action of the mediator, after which manifestations of the pain syndrome reappeared. In the control experiments microinjection of hypertonic NaCl solution gave no such effect (Fig. 1).

Glycine, if applied together with strychnine, did not prevent development of the pain syndrome. GABA lengthened the latent period of the pain syndrome when applied with strychnine, but later the syndrome increased rapidly, to end simultaneously with the end of the syndrome in the control animals. The duration of the action of GABA when applied together with strychnine, incidentally, was shorter than when applied with tetanus toxin.

Glycine, if applied together with penicillin, prevented development of the pain syndrome: The syndrome completely failed to appear, evidently because the duration of the effect of glycine was the same as that of penicillin. GABA did not prolong the latent period of onset of the pain syndrome during the action of penicillin, but reduced the duration of the syndrome by half.

The effects of the mediators were similar in type when applied together with KCl or ouabain; both mediators prevented the development of the pain syndrome, and the duration of their action evidently coincided with the duration of action of the depolarizing agents, so that under these conditions the pain syndrome did not develop.

The data given above thus show that although GPEEs induced by pharmacological agents of different nature (disinhibitors and depolarizing agents) led to the appearance of a similar symptom-complex characteristic of a pain syndrome of spinal origin [1, 2, 4], the action of the inhibitory mediators depended on the nature of the generator produced. Glycine is known to cause hyperpolarization of the postsynaptic membrane [9, 12], and it competes with strychnine for receptors on the membrane [11, 16]. Glycine therefore was not effective against a GPEE created by strychnine. However, it was effective (preventing the development of the pain syndrome) in cases when the development of GPEE was not accompanied by blocking of the glycine receptors on the postsynaptic neuron membrane, i.e., when the GPEE was produced by means of tetanus toxin, penicillin, KCl, and ouabain.

GABA was effective against "tetanus," "potassium," and "ouabain" generators and partly effective against "strychnine" generators, i.e., when its receptors on the neuron membrane were not blocked [10, 12, 13, 15]. However, it was also partly effective against the "penicillin" generator, when the GABA receptors on the neuron membrane were blocked.

The facts described above show that neurons of the posterior horn, belonging to the nociceptive system, are sensitive both to glycine and to GABA, a fact which probably increases the reliability of the inhibitory apparatus.

By depressing GPEEs created in the system of nociceptive sensation by inhibitory mediators it is thus possible to abolish the whole syndrome during the action of the mediator. These findings agree with the results of other investigations which showed that trigeminal neuralgia can be relieved by injecting glycine into the caudal nucleus of the spinal tract of the trigeminal nerve, in the region of location of a GPEE created with tetanus toxin [6]. Under these conditions inhibitory mediators produce an analgesic effect, although they are not analgesics in the pharmacological sense. Administration of GABA and glycine may be a valuable method of investigating pathogenetic mechanisms and, in particular, the generator mechanisms of central pain, with the aim of investigating the possibility of its relief.

LITERATURE CITED

1. V. N. Grafova and E. I. Danilova, in: Problems in the General Theory of Disease. Collection of Scientific Works [in Russian], No. 1, Moscow (1976), pp. 113-116.
2. G. N. Kryzhanovsky (G. N. Kryzhanovskii), in: Advances in Pain Research and Therapy, edited by J. M. R. Besson et al., Vol. 1, New York (1976), pp. 225-230.
3. G. N. Kryzhanovskii, Zh. Nevropatol. Psikhiat., No. 11, 1730 (1976).
4. G. N. Kryzhanovskii, V. N. Grafova, E. I. Danilova, et al., Byull. Éksp. Biol. Med., No. 7, 15 (1974).
5. G. N. Kryzhanovskii and S. I. Igon'kina, Byull. Éksp. Biol. Med., No. 6, 651 (1976).
6. G. N. Kryzhanovskii, S. I. Igon'kina, V. N. Grafova, et al., Byull. Éksp. Biol. Med., No. 11, 16 (1974).
7. G. N. Kryzhanovsky (G. N. Kryzhanovskii) and F. D. Sheikhon, J. Exp. Neurol., 50, 387 (1976).
8. D. R. Curtis and W. Z. De Groat, Brain Res., 10, 208 (1968).
9. D. R. Curtis and G. A. R. Johnston, Ergebn. Physiol., 69, 98 (1974).
10. R. A. Davidoff, Brain Res., 45, 638 (1972).
11. J. J. Dreifuss and E. R. Matthews, Brain Res., 45, 599 (1972).
12. R. Evans, A. Francis, and F. Watkins, Brain Res., 118, 395 (1976).
13. A. A. Fedinec and R. Shank, J. Neurochem., 18, 2222 (1971).
14. R. S. Lee and W. Klaus, Pharmacol. Rev., 23, 193 (1971).
15. E. Roberts, T. N. Chase, and T. B. Tower (editors), GABA in Nervous System Function, New York (1976).
16. A. K. Tebecis and A. Di Maria, Brain Res., 40, 373 (1972).

EFFECT OF HYPEROXIA AND THE PROTECTIVE ACTION OF UREA ON SERUM HEMOGLOBIN, TRANSFERRIN, AND TOTAL IRON CONCENTRATIONS

V. V. Vnukov, A. A. Krichevskaya, and A. I. Lukash

UDC 612.273.1

During exposure of albino rats to oxygen under a pressure of 4 atm for 1 h (the preconvulsive state) and of 6 atm (convulsive state) an increase in the serum hemoglobin concentration was found. The total ion concentration increased at the same time. By disc electrophoresis in 7.5% polyacrylamide gel, changes in the ratio between the hemoglobin fractions in the serum were found in both stages of oxygen poisoning. An increase in the concentration of transferrins was found during hyperoxia under these conditions. If urea was administered to the animals before the session of hyperbaric oxygenation, the changes observed were less marked.

KEY WORDS: hyperoxia; blood serum; hemoglobin; total iron; transferrins.

Oxygen under increased pressure (hyperbaric oxygenation) is widely used in medicine and in various types of occupation. One of the complications encountered during the use of hyperbaric oxygenation in clinical practice is oxygen poisoning.

The first link in the chain of reactions in oxygen poisoning is the accumulation of free-radical and peroxide compounds. Many factors considerably potentiate the primary hyperoxic effect. In the writers' view, the escape of hemoglobin from the erythrocytes into the blood serum and its penetration into the tissues is one such factor. This is because of the ability of hemin iron to catalyze peroxidation of lipids [1].

In the investigation described below the hemoglobin concentration, its fractional composition, and the total concentrations of iron and transferrins were studied in the blood serum of rats exposed to the action of

Department of Biochemistry, Rostov-on-Don University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 6, pp. 528-531, June, 1979. Original article submitted May 19, 1978.

 0007-4888/79/8706-0548$07.50