CONNECTION BETWEEN CENTRAL ACTION OF GLYCERYL TRINITRATE AND MONOAMINE METABOLISM OF THE BRAIN

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We have previously shown that glyceryl trinitrate depresses reflex reactions of the coronary vessels and arterial pressure in response to stimulation of various reflexogenic zones and that this effect is due to the influence of the drug on the centers controlling the circulation [2, 3]. Comparison of these findings with observations of other authors showing that glyceryl trinitrate does not increase the blood flow in the vessels of the heart [7, 12] led to the suggestion that its clinical value may be determined by its central action. This hypothesis, however, indicates only in general features the properties of glyceryl trinitrate by virtue of which it remains even today the most reliable remedy for the treatment of angina pectoris.

The object of the present investigation was to continue the study of the mechanism of action of glyceryl trinitrate on processes of central regulation of vascular tone.

EXPERIMENTAL METHOD

Experiments were carried out on cats anesthetized with urethane (200-300 mg/kg) and chloralose (20-50 mg/kg). Since constrictor reflexes acting on the coronary vessels are mediated through the sympathetic innervation of the heart [3], in order to judge the effect of glyceryl trinitrate on the central processes responsible for formation of these reflexes, the method of electroneurographic recording of tonic and reflex activity in the inferior cardiac nerve during electrical stimulation of the central end of the divided tibial nerve, with differentiation of responses to impulses in A and C groups of afferent fibers was used [6]. To judge the effect of glyceryl trinitrate on reflex inhibition of tonic activity in the sympathetic nerves of the heart, the mechanoreceptors of the carotid sinus were stimulated by raising the pressure. This was done by pumping blood from the peripheral end of the animal's divided carotid artery into the region of the carotid sinus, which was isolated from the systemic circulation, at assigned perfusion pressures.

In a special series of experiments the noradrenalin concentration was determined in various parts of the cats' brain by a spectrofluorometric method [11].

EXPERIMENTAL RESULTS AND DISCUSSION

These experiments showed that glyceryl trinitrate in a dose of 1 mg/kg depresses spontaneous electrical activity and reflex discharges in the inferior cardiac nerve. In some experiments selectivity of its action was observed in relation to reflex discharges from afferent C-fibers along which nociceptive impulses are known to be transmitted into the central nervous system. At the same time pressor vasomotor reflexes were depressed. The effect lasted for 30-35 min (Fig. 1).

An interesting fact revealed by our previous investigations was that similar changes in character of the reflex discharges in the sympathetic nerves develop under the influence of monoamines and of their precursors—DOPA and 5-hydroxytroptophan, and also of substances capable of liberating monoamines from tissue reserves: reserpine (in phase I of its action), analgesics, and MAO inhibitors [4]. These substances simultaneously caused a sharp increase in the intensity of reflex inhibition of tonic activity evoked by stimulation of the carotid sinus mechanoreceptors. The development of these effects coincides in time with the period of liberation of monoamines from reserves of the labile fraction [4, 5, 8, 13, 14]. After comparing these observations with the histochemical findings of Carlson and co-workers [9], who found a system of

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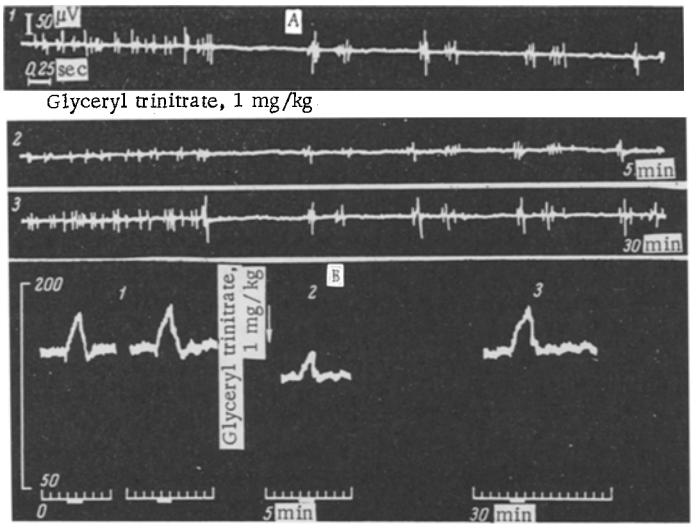

Fig. 1. Effect of glyceryl trinitrate on tonic activity and reflex discharges in the inferior cardiac nerve in response to impulses from afferent fibers of A and C groups and vasomotor reflexes arising during electrical stimulation of afferent fibers of the tibial nerve. A) Tracing of tonic activity and reflex discharges in inferior cardiac nerve during electrical stimulation of central end of divided tibial nerve (3 V, 2 msec, 1 stimulus/sec); B) tracing of vasomotor reflexes arising during electrical stimulation of central end of divided tibial nerve (30 V, 1 msec, 30 stimuli/sec for 20 sec); 1) tonic activity and reflex responses in inferior cardiac nerve and vasomotor reflexes before injection of glyceryl trinitrate; 2) the same 5-7 min after injection of glyceryl trinitrate in dose of 1 mg/kg body weight; 3) the same 30 min after injection of the drug.

Effect of Glyceryl Trinitrate (1 mg/kg) on Noradrenalin Concentration (in μg/g) in Different Parts of the Brain Tissue in Cats*

Time of observation	Hypothalamus	Medulla	Thoracic portion of spinal cord
Control	0,55±0,077	0,63±0,11	0,5±0,09
30 Min after injection	0,24±0,04	0,2±0,017	0,27±0,08
2 h after	0,29±0,04	0,3±0,05	0,37±0,006
4 h after	0,47±0,12	0,5±0,1	0,4±0,07

*Mean results of the series of experiments with standard error are shown in the table.

inhibitory monoaminergic neurons descending from the vasodepressor region of the medulla in the posterolateral columns of the spinal cord, we were able to postulate that the observed effects are associated with activation of the inhibitory system of monoaminergic neurons by monoamines.

It was stated above that the character of the action of glyceryl trinitrate on tonic and reflex activity of the sympathetic nervous system is analogous to the effects of monoamines and of substances liberating their tissue reserves just described. However, this was not the only factor suggesting that central adrenergic mechanisms are concerned in the development of the effects of glyceryl trinitrate. This compound is known to abolish symptoms of excitation of the sympathetic nervous system arising during attacks of angina pectoris. The view is also held that its clinical efficacy is associated with its ability to abolish sympathetic influences on the myocardium and thereby to reduce the energy requirements of the heart [10, 15, 16].

The study of the action of glyceryl trinitrate in experiments in which injury to the myocardium was produced by injection of potassium chloride into the lateral ventricle of the brain showed that the drug abolishes the increased bioelectrical activity developing under these conditions in the sympathetic nerves of the heart and prevents accumulation of catecholamines in the myocardium [1]. On the basis of these findings, we postulated that adrenergic mechanisms may be concerned in the mechanism of action of

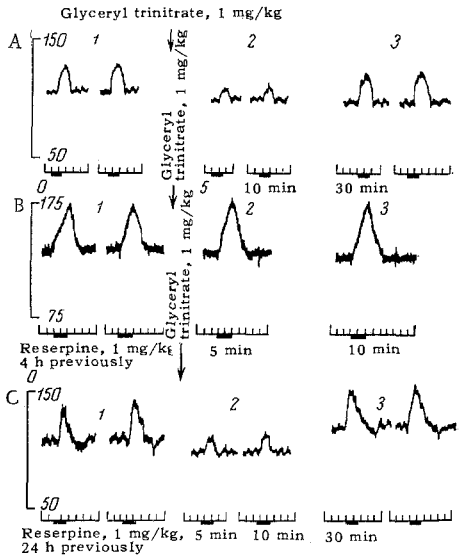

Fig. 2. Effect of glyceryl trinitrate in a dose of 1 mg/kg body weight on vasomotor reflexes against the background of reserpine action. A) Effect of glyceryl trinitrate on vasomotor reflexes arising during electrical stimulation of central end of divided tibial nerve; B) effect of glyceryl trinitrate on vasomotor reflexes arising during electrical stimulation of central end of divided tibial nerve (20 V, 2 msec, 15 stimuli/sec for 20 sec) 4 h after administration of reserpine (1 mg/kg); C) effect of glyceryl trinitrate on vasomotor reflexes arising during electrical stimulation of central end of divided tibial nerve (25 V, 1 msec, 30 stimuli/sec for 15 sec) 24 h after injection of reserpine (1 mg/kg).

glyceryl trinitrate on the centers regulating the circulation. To verify this hypothesis it was first necessary to show whether the action of glyceryl trinitrate is exhibited against the background of changes in the monoamine concentration in brain tissue produced by reserpine. In a special series of experiments we investigated the effect of glyceryl trinitrate on vasomotor reflexes 4 h after injection of reserpine. This interval was deliberately chosen. According to data in the literature [14], in this period of time reserpine completely exhausts the labile fraction of the monoamine reserves. This view regarding the dynamics of reserpine action in relation to vasomotor responses was confirmed in the previous investigations of one of us (Yu. B. R. [8]). Experiments in which glyceryl trinitrate was given against the background of reserpine confirmed that its action is dependent on monoamine liberation. When glyceryl trinitrate was injected 4 h after reserpine (1 mg/kg), it did not change the intensity of the vasomotor reflexes. Different results were obtained 24 h after injection of reserpine, i.e., when, despite the low total monoamine level, their labile reserve had been restored [14]. At this time glyceryl trinitrate, like other substances whose action is due to an increase in labile forms of monoamines, again inhibited the vasomotor reflexes (Fig. 2). It was concluded from these experiments that the action of glyceryl trinitrate is mediated and effected through labile, functionally active, forms of monoamines.

As a result of pharmacological action, besides a redistribution of monoamines leading to predominance of free forms, the enzymic breakdown of these substances is intensified, causing gradual depletion of their reserves. The next stage of the argument was accordingly as follows. If the effects of glyceryl trinitrate which we observed were due to an increase in free forms of monoamines in the brain tissue, under the influence of the drug the total monoamine content should fall gradually. This prediction was verified experimentally. The concentration of noradrenalin in the hypothalamus, medulla, and thoracic portion of the spinal cord was found to be considerably reduced 30 min after injection of glyceryl trinitrate (1 mg/kg). The results of this series of experiments are given in the table.

As the table shows, 30 min after injection of glyceryl trinitrate the noradrenalin concentration in the brain tissue was considerably reduced (by 50-70% compared with its initial level). A tendency toward recovery was found after 2 h, and after 4 h the noradrenalin concentration was close to its original level.

The results of these experiments thus confirmed our hypothesis that the action of glyceryl trinitrate on the tonic and reflex activity of the sympathetic nervous system and the vasomotor reflexes is associated with its influence on monoamine metabolism in the central nervous system.

It was now essential to identify the central processes with action on which the effect of glyceryl trinitrate is associated and to determine whether the effect of the drug is due to activation of inhibitory monoaminergic mechanisms. To examine this problem, in a special series of experiments we investigated the effect of glyceryl trinitrate on reflex inhibition of tonic activity in sympathetic nerves and also on depressor reflexes evoked by stimulation of the carotid sinus mechanoreceptors. This reflex, as we know, is effected through the vasodepressor region of the medulla. The experiments showed that 5 min after injection of glyceryl trinitrate the intensity and duration of reflex inhibition of tonic activity in the sympathetic nerves of the heart rose sharply and the intensity of the depressor reflex was considerably increased. The effect lasted for 30-35 min.

The mechanism of the central action of glyceryl trinitrate on tonic and reflex activity of the sympathetic nervous system and on vasomotor reflexes is thus associated with its influence on metabolism of the biogenic monoamines. A temporary increase in the concentration of free, functionally active, forms of monoamines apparently causes activation of the inhibitory monoaminergic neurons. The predominance of inhibitory over excitatory processes taking place in these circumstances may be the basis for the central depriming effects of the monoamines.

It may be concluded from a comparison of these data with our previous observations [4] that the mechanism described above is common to a number of pharmacological substances capable of increasing, for a definite period of their action, the concentration of free monoamines in brain tissue.

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