TYROSINE HYDROXYLASE ACTIVITY IN AUTONOMIC GANGLIA OF RABBITS WITH ACUTE EXPERIMENTAL EMOTIONAL STRESS

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Previous investigations [3] showed that in rabbits during emotional stress metabolic activity is changed in structures of both the sympathetic system and the vagus nerve system, but not to the same degree in rabbits predisposed to emotional stress and rabbits resistant to its development. In rabbits predisposed to stress, catabolic processes in the ganglion nodosum predominate over anabolic (the content of water-soluble proteins and structural proteins in nerve cell nuclei is reduced). In rabbits resistant to stress, a significant increase is observed in the content of water-soluble protein in homogenates of the ganglion nodosum compared with the control. In homogenates of the superior cervical ganglion, the content of water-soluble proteins did not differ from the control, but in homogenates of the sympathetic chain and stellate ganglion it was increased.

The aim of the present investigation was to discover what changes take place in the activity of structures of the sympathetic and parasympathetic systems in the course of a 3-hour experiment, with particular reference to the study of tyrosine hydroxylase activity, for activity of this enzyme is known to correlate with the functional state of the structure containing it.

EXPERIMENTAL METHOD

Emotional stress was indeed induced in immobilized rabbits by simultaneous aperiodic stimulation of negative emotiogenic centers of the hypothalamus (ventromedial nuclei) and electrodermal stimulation by a specially devised stochastic scheme. The ventromedial hypothalamic nuclei were stimulated by bipolar nichrome electrodes, and electric shocks were applied to the skin through steel needles, inserted beneath the skin of one of the animal's hind limbs. Square pulses of current with a frequency of 50 Hz, duration 1 msec, and voltage 5-10 V were used in the experiments and the duration of stimulation was 1-2 min. Parameters of the electric current were chosen individually and were calculated to raise the arterial pressure (BP) by 20-30 mm Hg without inducing any marked motor response of the animal. Throughout the performance of the whole stress program (3 h), BP was recorded continuously in the rabbits by means of strain guage and piezoelectric transducers, connected to a Mingograph (Siemens-Elema, Sweden). Depending on the character of the changes in BP, the experimental animals were divided into two groups: resistant and predisposed to stress. The group of resistant animals was characterized by very small fluctuations in BP, whereas in the group of predisposed animals, BP fell progressively until death, which occurred 2 h 15 min-2 h 50 min after the beginning of the experiments. The ganglion nodosum (parasympathetic) and the cervical and stellate (sympathetic) ganglia were removed immediately after death of the rabbits predisposed to stress. The same structures also were taken from rabbits killed by air embolism 30 and 60-90 min and 3 h after the beginning of the experiment. Animals kept under ordinary animal house conditions served as the control.

Each ganglion (weighing from 2 to 8 mg) was homogenized in 80 µl of 5 mM Tris-buffer (pH 6.0), containing 0.1% Triton X-100, and centrifuged for 30 min at 5000g. Tyrosine hydroxylase activity was determined in 10 µl of supernatant by the method in [1]. Samples were incubated for 4 min at 37°C in 0.1 M phosphate buffer (pH 6.0) in the presence of 80 µM [^{3}H]tyrosine

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TABLE 1. Tyrosine Hydroxylase Activity in Ganglia of Autonomic Nervous System (in pmoles Dopa/mg protein/min)

Ganglion	Control (n = 27)	30 min of experiment (n = 6)	P	60-90 min of experiment (n = 6)	P	Rabbits predisposed to stress (n = 7)	P	Rabbits resistant to stress (n = 4)	P
Superior cervical	178±9	122±12	<0,01	217±18	>0,05	91±12	<0,001	124±16	<0,05
Nodosum	93±7	115±52		204±33	<0,001	26±8	<0,001	100±15	
Stellate	177±15	111±20	<0,05	245±20	>0,05	61±15	<0,001	196±49	

(specific activity 13.1 mCi/mmole, USSR), 0.7 mM 6,7-dimethyl-5,6,7,8-tetrahydropterine-HCl (from Serva, West Germany), and 0.3 M mercaptoethanol (also from Serva). The total volume of the incubation sample was 20 µl. The reaction was stopped by the addition of 250 µl of 0.4 N $HClO_4$ containing 0.4 µg of unlabeled dopa (from Serva). [^{3}H]Dopa was separated from [^{3}H]tyrosine on a column with Al_2O_3 (D = 7 mm) chromatographically, and it was then washed successively with 5 and 15 ml of 0.005 M Tris-buffer, pH 8.6. The [^{3}H]Dopa was eluted with 1.5 ml of 0.5 M CH_3COOH and 1 ml of eluate was added to 10 ml of scintillation fluid (composition of 1 liter: toluene 666 ml, Triton X-100 333 ml, PPO 4 g, POPOP 0.1 g). Tyrosine hydroxylase activity was expressed in picomoles dopa/mg protein/min. The experimental samples contained from 2 to 10 mg protein/ml. The protein content was determined as in [11]. All the numerical results were subjected to statistical analysis by the Student–Fisher method.

EXPERIMENTAL RESULTS

Tyrosine hydroxylase activity 30 min after the beginning of the experiment was reduced in ganglia of the sympathetic nervous system (cervical and stellate) by 32 and 38% respectively compared with the control (Table 1). In the ganglion nodosum tyrosine hydroxylase activity was indistinguishable from the control level. An increase in tyrosine hydroxylase activity by 22% was found in the cervical and stellate ganglia 1.5 h after the beginning of the experiment, and an increase by 128% in the ganglion nodosum. In rabbits predisposed to stress, tyrosine hydroxylase activity was significantly below the control level in all ganglia studied — by 50, 66, and 72% respectively. Tyrosine hydroxylase activity in the cervical ganglion of rabbits resistant to stress was 30% lower than in the control animals, whereas in the stellate ganglion and ganglion nodosum it was the same as in the control.

Increased activity of the sympathico-adrenal system in emotional stress can now be regarded as firmly established [4, 5]. A marked increase in the blood catecholamine levels is observed under these conditions [4, 5, 8]. Catecholamines and, in particular, noradrenalin, the mediator of the sympathico-adrenal system, are important regulators of the adaptive reactions of the body. They enable the body to switch quickly and adequately from a state of rest into a state of excitation and to remain in that state for a long time. An important role in the regulation of catecholaminergic processes is played by tyrosine hydroxylase, the key enzyme of catecholamine biosynthesis [7, 13]. Tyrosine hydroxylase catalyses oxidative conversion of tyrosine into dopa. This is the slowest reaction in the chain of reactions of catecholamine biosynthesis and it determines the overall velocity of this process. Tyrosine hydroxylase activity in various brain structures correlates with the level of their physiological activity. In the present investigations unequal tyrosine hydroxylase activity was found in the course of a 3-hour experiment in ganglia both of the sympathetic system and of the vagus nerve system. Changes in physiological activity of ganglia of the sympathetic nervous system (cervical and stellate), manifested as a fall in tyrosine hydroxylase activity, in all probability occurred 30 min after the beginning of the experiment. Noradrenalin biosynthesis, as we know, is a classical example of regulation by negative feedback type [2], when the limiting enzymic stage of biosynthesis is regulated by a change in concentration of the end product (noradrenalin). Noradrenalin regulates the rate of catecholamine biosynthesis by inhibiting tyrosine hydroxylase. It can be tentatively suggested that under these conditions, when in all probability the blood noradrenalin level was raised and, in addition, release of noradrenalin from terminals of adrenergic neurons was intensified, active uptake of noradrenalin took place by the cell surface of adrenergic neurons from the extracellular space [9], with the result that the intracellular noradrenalin concentration rose. This, in turn, could lead to modification of the enzyme [7], manifested in the present experiments as a fall in tyrosine hydroxylase activity. Its activity in the parasympathetic ganglion (ganglion nodosum) at this time did not differ significantly from the control. The increase in tyrosine hydroxylase ac-

tivity observed 1.5 h after the beginning of the experiment in the ganglion nodosum may perhaps indicate an increase in physiological activity of the parasympathetic system. One possible way of regulating the activity of the enzyme at this time is by its transition from the soluble to the membrane-bound form, in which the enzyme is more active [7, 10]. The absence of changes in tyrosine hydroxylase activity in the stellate ganglion of the sympathetic system and in the ganglion nodosum, of the vagus nerve system, observed toward the end of a 3-hour experiment in rabbits resistant to stress indicates, in all probability, the development of a state of adaptation to stress. In rabbits predisposed to stress, we may suppose, the functional capacity of the ganglion nodosum was overstrained, as shown under these experimental conditions by a fall in tyrosine hydroxylase activity. The fall in tyrosine hydroxylase activity in the stellate ganglion was probably the result of an increase in the noradrenalin concentration in the neuron bodies as a result of neuronal uptake. This hypothesis is supported by data of Portugalov et al. [6], who observed a fall in the noradrenalin content to zero in the ganglion nodosum and an increase in its content in the stellate ganglion toward the end of 3 h of stress.

With reference to tyrosine hydroxylase activity, these experiments thus demonstrated that changes in metabolic activity of the sympathetic and parasympathetic systems in the course of an experiment lasting 3 h take place in stages; the character of the changes in tyrosine hydroxylase activity was found to differ in the ganglion nodosum and stellate ganglion of rabbits predisposed and resistant to stress: in rabbits predisposed to stress, tyrosine hydroxylase activity fell in the stellate ganglion and ganglion nodosum.

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