tem [10]. We believe that the administration of FMRFa-like peptides stimulates this system and activates both the sympatho-adrenal axis and, probably, an as yet unstudied reflex component responsible for positive shifts in respiratory and cardiovascular functions during reanimation.

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# Effect of Histidine-Containing Dipeptides on Brain Tyrosine Hydroxylase

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Histidine-containing dipeptides inhibit highly purified tyrosine hydroxylase. The degree of inhibition is dependent on the peptide structure and decreases according to the following sequence: carnosine>anserine>homocarnosine. This effect is not associated with the buffer and antioxidant properties of the dipeptide.

**Key Words:** *tyrosine hydroxylase; antioxidants; carnosine; anserine; homocarnosine*

The histidine-containing dipeptides carnosine and its analogs display a diverse physiological and biochemical activity [5,8]. However, the molecular mechanisms of the effects of these compounds are not fully understood. Histidine-containing peptides fulfill two major functions in the cell: they act as a buffer, facilitating the stabilization of the intracellular pH [6], and as an endogenous antioxidant, protecting membranes against the damaging effect of lipid peroxidation products [1].

However, the recently reported localization of both tyrosine hydroxylase (TH) and carnosine in neurons [7] cannot be explained solely by these functions.

Tyrosine hydroxylase (tyrosine-3-monoxygenase, EC 1.14.16.2) catalyzes the first step of catecholamine biosynthesis: the hydroxylation of L-tyrosine to form L-DOPA. In animals, L-DOPA is probably not involved in plastic processes and fulfills a specific function, being a catecholamine precursor. The TH reaction is the limiting stage of catecholamine biosynthesis [10], which determines its importance for normal functioning of the nervous system. A complex and rapid regulation of TH enables this functionally important enzyme to adapt to the rapidly changing intracellular environment. Allosteric regulation is a means of rapid modulation of the enzyme activity [9]. Allosteric properties of TH have been demonstrated with pterin co-

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factor, substrates (L-tyrosine and oxygen), reaction product (L-DOPA), and retroinhibitors (catecholamines). The enzyme interacts with the tetrapeptide tuftsin, an immunoglobulin G fragment [2] containing proline, which is a remote analog of histidine in terms of electron structure.

In the present study we compared the direct effect of carnosine and its analogs on TH isolated from rat hypothalamus with the effects of structurally different antioxidants.

## MATERIALS AND METHODS

Tyrosine hydroxylase was purified from rat hypothalamus by affinity chromatography on diiodothyronine-Sepharose-4B as described elsewhere [4]. The enzyme fraction with a molecular weight of 36 kD (monomeric TH) was used in experiments. The rate of the TH reaction was measured by direct spectrophotometry [3] in a double-beam mode from the increase in light absorbance at 335 nm. The incubation medium contained Tris-maleate (pH 6.15), enzyme (1 μg/ml sample), L-tyrosine (9 μM), and 6,7-dimethyl-5,6,7,8-tetrahydropterin ($DMPH_4$, 147 μM). The antioxidants were added in a concentration of $10^{-6}$ M.

The following reagents were used: Tris-base, L-tyrosine, dithiothreitol, 2-mercaptoethanol, anserine (nitrate), homocarnosine (Sigma), carnosine (St. Petersburg Plant of Medical Preparations), $DMPH_4$ (recrystallized from an ethanol/acetone mixture, Calbiochem), maleic acid (recrystallized three times, Erevan Plant of Chemical Reagents), Russian-manufactured emoxypine, and hydrochloric acid ("high grade") for the preparation of L-tyrosine and $DMPH_4$ solutions.

## RESULTS

Table 1 shows the rates of the TH reaction measured in the presence of anserine, carnosine, and homocarnosine. The dipeptides were added to the cuvette after the reaction rate had been recorded for 1 min, i.e., to the complete system consisting of the enzyme, substrate (L-tyrosine), and pterin cofactor. After the addition of peptides, the reaction remained linear, although its rate changed. It can be seen from the table that the peptides inhibited TH in a concentration-dependent manner. Carnosine produced the most potent inhibiting effect: in a concentration of 25 μM it lowered the reaction rate to 20% of the initial rate measured in the same sample prior to the addition of the dipeptide. The concentrations at which the dipeptides elicit an inhibitory effect on TH are comparable to the $K_m$ for tyrosine, which is of the order of $10^{-5}$ M.

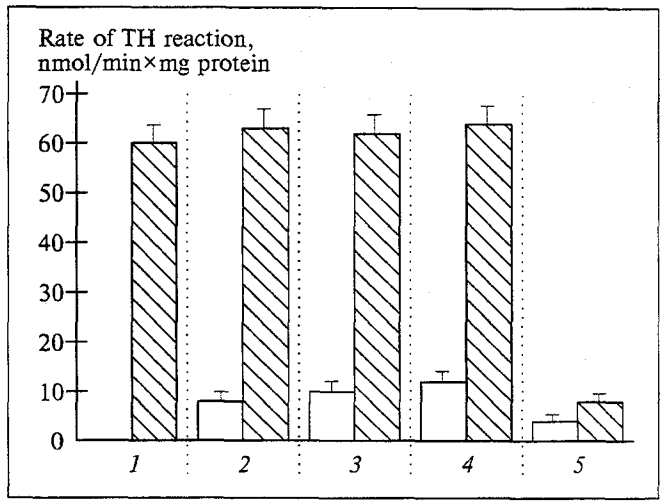


**Fig. 1.** Inhibition of tyrosine hydroxylase (TH) activity by different antioxidants depending on the time of their addition to the reaction mixture. *1*) control; *2*) emoxypine ($10^{-6}$ M); *3*) dithiothreitol ($10^{-6}$ M); *4*) 2-mercaptoethanol ($10^{-6}$ M); *5*) carnosine ($2 \times 10^{-5}$ M). White bars: antioxidant added before tyrosine; shaded bars: antioxidant added after tyrosine.

Thus, we have shown that the histidine-containing dipeptides anserine, carnosine, and homocarnosine display an affinity for TH that depends on the dipeptide structure.

The TH reaction was carried out in a Tris-maleate buffer, which has a high buffer capacity at pH 6.2, since this pH is consistent with the pK of maleate. The pH of the reaction mixture remained stable in the presence of the dipeptides, and therefore their direct effect on TH *in vitro* cannot be associated with their buffer properties.

The antioxidant activity of the dipeptides during inhibition of TH was assessed in experiments with antioxidants differing in structure (Fig. 1). The thiol antioxidants dithiothreitol and mercaptoethanol and the 3-hydroxypyridine antioxidant emoxypine produced a pronounced inhibitory effect only after being added to the incubation mixture before L-tyrosine (the enzyme substrate), after which the reaction rate dropped 7- to 8-fold. When added

**TABLE 1.** Effects of Histidine-Containing Dipeptides on the Rate of the Tyrosine Hydroxylase Reaction, % of Control

| Compound | Concentration, μM | Residual reaction rate, % |
|---|---|---|
| Anserine | 16 | 65 |
| | 25 | 54 |
| Homocarnosine | 20 | 61.5 |
| Carnosine | 20 | 26 |
| | 25 | 19.6 |

**Note.** The initial reaction rate in the control is 55±4 nmol/min×mg protein.

after tyrosine, i.e., after the formation of the enzyme-substrate complex, these antioxidants had no effect. On the contrary, in this case the reaction rate remained stable and high for a long time due to the presence of the antioxidants, which inhibited spontaneous (nonenzymatic) oxidation of pterin cofactor. The situation was different after the addition of carnosine. In a concentration of $5\times10^{-5}$ M carnosine inhibited the reaction before and after the addition of tyrosine (Fig. 1), the effect being 5-fold higher, when carnosine was added before tyrosine. This shows that carnosine acts on TH in a specific manner that is not associated with its antioxidant properties: in contrast to carnosine, typical antioxidants with different structures (emoxypine and thiol compounds) did not inhibit the reaction after being added to the enzyme-substrate complex.

The evidence that histidine-containing peptides interact with TH, a key enzyme of catecholamine biosynthesis, indicates that these peptides (specifically, carnosine) can act as factors of specific regulation of TH. Presumably, the effect of histidine-containing peptides is of an allosteric nature. From this viewpoint the interaction with TH can be regarded as a possible mechanism whereby histidine-containing enzymes are involved in the maintenance of the specific function of neurons.

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