ACETYLCHOLINE METABOLISM IN THE BRAIN OF RATS TREATED WITH VANADYL SULFATE AND CERTAIN PHENYLACETIC ACID DERIVATIVES

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The formation of acetylcholine (AC) in the brain involves the direct participation of coenzyme A [5, 10]. Many pharmacological agents are known which are capable of influencing this process in different ways. Among these may be mentioned vanadyl sulfate and certain derivatives of phenylacetic acid, for some of their pharmacological effects can be attributed to their action on the function of coenzyme A [2, 4, 8, 11]. The combined administration of these compounds is accompanied by various forms of synergism between them [2].

The object of the present study of certain aspects of AC metabolism (cholinesterase activity, concentration of "free" and "bound" AC) in the rat's brain was to obtain further elucidation of the mechanism of the combined action of these substances on the various biochemical reactions in the body involving coenzyme A.

EXPERIMENTAL METHOD

Rats were given an intraperitoneal injection of vanadyl sulfate ($VOSO_4 \cdot 2H_2O$) in a dose of 1-10 mg/kg 19-20 h before decapitation. One hour before sacrifice the animals received subcutaneous injections of phenylacetic (PA), phenylethylacetic (PEA), and diphenylacetic (DPA) acids in doses of 0.5 and 1.0 mM/kg. The total cholinesterase activity [12] and the "free" and "total" AC were determined. The difference between the "free" and "total" concentrations of AC gave the "bound" AC [1]. For determination of the AC level the test tissue was homogenized with the addition of eserine and extracted with Ringer's solution at pH 7.6 (1 : 10). For determination of the "free" AC the extraction was carried out in the cold (ice); for determination of the "total" AC the extract was heated on a boiling water bath for 5 min (to denature the proteins binding acetylcholine [1]). The extracts were tested on a preparation of the dorsal muscle of a leech [3]. In all the experiments parallel determinations were made of the protein concentration by the biuret reaction, and the ratios between the total cholinesterase activity and the values obtained for the "free" and "bound" AC, on the one hand, and the protein concentration, on the other, were calculated.

Three series of experiments were performed. In series 1 the "free" and "bound" AC in the brain and also the total cholinesterase activity were determined in animals receiving different doses of PA, PEA, and DPA; in series 2 these indices were determined in animals receiving vanadyl sulfate alone, and in series 3 – in animals receiving both vanadyl sulfate and the sodium salts of the test acids. Control animals received corresponding volumes of physiological saline.

EXPERIMENTAL RESULTS

The results given in the table show that administration of PA, PEA, and DPA to animals was accompanied by some decrease in the "total" AC in the brain, whereas the total cholinesterase activity of the tissues remained at its initial level (the changes observed were not statistically significant). The decrease in the "total" AC in these circumstances was most probably due to a decrease in the "bound" AC in the tissues, for the content of "free" AC not only did not fall but, on the contrary, it showed a tendency to increase.

Effect of Combined Administration of Certain PA Derivatives (Sodium Salts) and Vanadyl Sulfate (VS) on the Total Cholinesterase Activity and Content of "Total" and "Free" AC in the Rats' Brain

Experimental conditions*	Content of "total" AC (μg/mg protein)	Content of "free" AC (in μg/mg protein)	Total cholinesterase activity (in μg/mg/h of incubation)
Series 1 of experiments			
PA (0.5 mM/kg)	0.312 ± 0.043	0.09 ± 0.040	5.84 ± 0.41
PA (1 mM/kg)	0.273 ± 0.031	0.075 ± 0.031	5.70 ± 0.70
PEA (0.5 mM/kg)	0.278 ± 0.077	0.142 ± 0.04	5.85 ± 0.58
PEA (1 mM/kg)	0.229 ± 0.069	0.12 ± 0.035	6.22 ± 0.5
DPA (0.5 mM/kg)	0.242 ± 0.039	0.1 ± 0.056	5.7 ± 0.67
DPA (1 mM/kg)	0.19 ± 0.051	0.092 ± 0.021	5.68 ± 0.4
Series 2 of experiments			
VS (1 mg/kg)	0.323 ± 0.06	0.088 ± 0.032	5.77 ± 0.42
VS (10 mg/kg)	0.320 ± 0.07	0.096 ± 0.05	3.83 ± 0.7
Series 3 of experiments			
VS (10 mg/kg) + PA (0.5 mM/kg)	0.255 ± 0.04	0.081 ± 0.032	3.95 ± 0.41
VS (10 mg/kg) + PA (1 mM/kg)	0.225 ± 0.042	0.075 0.041	4.01 ± 0.32
VS (10 mg/kg) + PEA (0.5 mM/kg)	0.191 ± 0.075	0.126 ± 0.046	4.123 ± 0.56
VS (10 mg/kg) + PEA (1 mM/kg)	0.221 ± 0.05	0.321 ± 0.042	4.11 ± 0.48
VS (10 μ/kg) + DPA (0.5 mM/kg)	0.167 0.05	0.067 ± 0.07	4.12 ± 0.61
VS (10 μ/kg) + DPA (1 mM/kg)	0.158 ± 0.047	0.07 ± 0.03	3.82 ± 0.41
Control			
Physiological saline	0.448 ± 0.046	0.089 ± 0.56	5.76 ± 0.31

* Arithmetical mean values are compared, and differences treated statistically with P = 0.05 and t = 2.36.

All the acids investigated lowered the content of "bound" AC only, whereas the remaining "indices" of acetylcholine metabolism remained unchanged. Most active in this respect was DPA; PEA and PA were rather less active. Evidently the replacement of one of the hydrogen atoms attached to the carbon atom in the PA molecule by an ethyl or a heavier phenyl radical increases the effect observed in this series.

According to the literature [7] several other PA derivatives (diphenylylacetic, diphenylethane-ethylacetic acid) also have an inhibitory action on AC formation in the tissues. The action of PEA is slightly less marked; the activity of diphenylylacetic and diphenylethane-ethylacetic acids is close to that of PEA, PA, and DPA.

It can be assumed that the decrease in the "bound" AC as a result of the action of PA derivatives was due either to disturbance of AC synthesis (an effect on the function of coenzyme A) or to the action of the preparations on the stability of the bond between AC and reserve protein. In this case the first suggestion is more probable, for disturbance of the AC – protein complex could lead to a proportional increase in the "free" AC content. However, the facts did not confirm this. On the contrary, the content of "free" AC did not change in either direction with the same total cholinesterase activity. At the same time, these results also showed that the "reserve" AC content does not determine the content of "free" AC in the tissues by any means in every case.

The experiments showed that vanadyl sulfate depressed only the total cholinesterase activity and had no effect on the content of the "free" or "bound" AC in the tissues. In other words, despite the ability of vanadium ions to suppress the activity of coenzyme A [8], the content of "bound" AC (determined, in particular, by the activity of cholinacetylase and the activity of coenzyme A) was unchanged in this case. Meanwhile the total cholinesterase activity fell significantly. It may be suggested that the ability of the vanadium ions to influence the concentration and biosynthesis of substances containing SH-groups [7, 10] is exhibited to a greater degree, in the case of contact with a cholinergic structure, during interaction with its "cholinesterase component."

The results of the experiments in which PA derivatives and vanadyl sulfate were given together showed that the total cholinesterase activity of the brain tissues of the animals in this series was depressed to the same degree as when vanadyl sulfate was given alone. The content of "free" and "bound" AC was lower in all the experiments than, for example, when the PA derivatives were given separately (the difference observed in this series of experiments remained statistically not significant).

Hence, the combined administration of the compounds in this case had the result that the effects of PA and its derivatives were supplemented by the effect of vanadyl sulfate, inhibiting the total cholinesterase activity of the brain tissues. It might have been supposed that in these conditions the action of vanadyl sulfate would lead to a considerable increase in the constant of "free" AC, yet, as the experiments showed, this was not observed. It is possible that the interaction taking place in these experiments between the cholinesterases and the content of liberated AC was more complex and not characterized by a direct relationship.

It should be noted that the combined administration of vanadyl sulfate and a series of PA derivatives, by influencing the reaction of acetylation of sulfanilamides, leads to synergism of the "potentiating type," whereas in the reactions of acetylcholine metabolism as was observed in the present investigation, the effects of one component supplement the effects of the other. It appears that in the two reactions cited, in both of which coenzyme A plays a part, the investigated compounds and, in particular, vanadyl sulfate play a different role, determined by differences in the mechanism and in the point of their action.

SUMMARY

The present work describes the effect produced by phenylacetic acid, phenylethylacetic and diphenylacetic acids (sodium salts) separated and together with vanadyl sulfate on some "indices," of acetylcholine metabolism in the rat's brain. Total cholinesterase activity and the "free" and "bound" (conditionally) acetylcholine levels served as indices. As shown experimentally the use of phenylacetic acid derivatives is accompanied by reduction of the "bound" acetylcholine, content, whereas that of "free" acetylcholine and the total tissue cholinesterase activity remain unchanged. Vanadyl sulfate provokes a significant reduction of the total tissue cholinesterasic activity, but does not change the content of "free" and "bound" acetylcholine therein. In conjoint action of the above-mentioned substances, the effects of phenylacetic acid derivatives are supplemented by the anticholinesterase effect of vanadyl sulfate.

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