Cholinesterase Reactivators - Contribution to the Study of their Metabolic Effects

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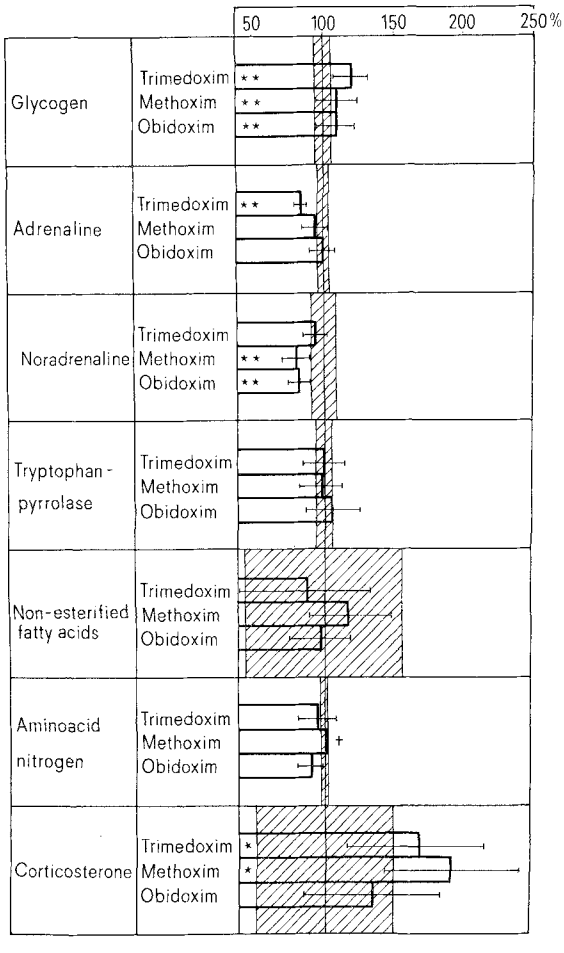
Summary. Cholinesterase reactivators – trimedoxim, methoxim and obidoxim – injected in the dose of 20 mg/kg s.c., increase muscle glycogen concentration in normal, but not in adrenalectomized rats. This effect may be in connection with simoultaneously found rise of serum corticosteron level. Trimedoxim decreases adrenaline, methoxim and obidoxim noradrenaline concentration in adrenals.

Cholinesterase (CHE) reactivators, drugs used in treatment of organophosphate poisoning, have been studied up to now for their antidotal potency. In our previous study, investigating their general pharmacological effects, we have shown that 3 tested CHE reactivators trimedoxim (TMB-4), obidoxim (LüH6, toxogonin) and methoxim (MMB-4) injected in to rats at a dose of 20 mg/kg i.p. increase liver and muscle glycogen concentration and the blood glucose level as well (BENEŠOVÁ and HVIZDOŠOVÁ[1]). This effect appeared as early as 45 min after the drug administration, and even higher and persistent values were attained readily after 2 h. To explain the mechanism of this action, we decided to investigate metabolic changes induced by CHE reactivators in more detail. This paper reports the results of a study concerning a possible involvement of adrenals (secretion of catecholamines and corticosteron) in rats. In addition, release of nonesterified fatty acids, activity of liver tryptophane pyrrolase and the blood level of aminoacid nitrogen were studied.



Concentration of glycogen in musculus biceps brachii (n = 41), concentration of adrenaline and noradrenaline in adrenals (n = 41), activity of liver tryptophane-pyrrolase (n = 41), concentration of non-esterified fatty acids (n = 22), amino acid nitrogen (n = 15) and corticosteron (n = 41) in blood serum of rats 2 h after the administration of trimedoxim, methoxim and obidoxim in doses of 20 mg/kg s.c. The results are expressed in percent of control values (saline) which are denoted by dashed zones (mean + 95% confidence limits). Bars in columns represent 95% confidence limits. Asterisks indicate statistically significant difference from control values ($p < 0.01$).
+ Due to the lack of blood serum, the estimation could be performed in 2 rats only.

Methods. Adult Wistar rats, fed standard pellet diet were used. All biochemical analyses were performed after 15 h food deprivation, water was given ad libitum. The CHE reactivators trimedoxim, methoxim and obidoxim were administered at a dose of 20 mg/kg s.c. 2 h before decapitation of the animals.

The following biochemical analysis were performed: the determination of glycogen in musculus biceps brachii and heart muscle (LUŠTINEC[2]), concentration of adrenaline and noradrenaline in adrenals (CHANG[3]), activity of tryptophane-pyrrolase in liver (KNOX[4]), serum corticosterone level by the modified method described by SILVER et al.[5], serum level of non-esterified fatty acids (NOVÁK[6]) and aminocid nitrogen (SOBEL et al.[7]).

In the last series of experiments, 18 epinephrectomized male Wistar rats (weight 200–250 g) were used; 24 h after epinephrectomy, they were treated with saline (n = 4), trimedoxim (n = 5), methoxim (n = 4) and obidoxim (n = 5), and 2 h later the determination of heart muscle glycogen (LUŠTINEC[2]) was performed.

All results were statistically evaluated by analysis of variance, statistical significance was plotted using Duncan test and t-test.

Results. The results of the first series of experiments are illustrated in the Figure. All 3 CHE reactivators tested increase significantly the muscle concentration of glycogen. This finding confirms our previously published results (BENEŠOVÁ and HVIZDOŠOVÁ[1]). The CHE reactivators also induce changes in catecholamines concentration in adrenals which are not of the same character. Trimedoxim decreases significantly the concentration of adrenaline, but does not change that of noradrenaline. Methoxim and obidoxim have just the opposite effect. None of the 3 CHE reactivators change the activity of liver tryptophane-pyrrolase.

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No changes in the levels of non-esterified fatty acids and aminoacid nitrogen levels in blood serum were found. All CHE reactivators increase the secretion of corticosterone. This effect reaches statistical significance with methoxim and obidoxim.

After epinephrectomy, CHE reactivators no longer evoked an increase in glycogen concentration in heart muscle, but rather a trend to decrease was seen, but this change was not significant (Table).

Glycogen concentration in heart muscle from normal and epinephrectomized rats 2 h after administration of drugs (20 mg/kg s.c.)

Treatment	Normal rats			Epinephrectomized rats		
	n	mg/100 g	$s_{\bar{x}}$	n	mg/100 g	$s_{\bar{x}}$
Saline	43	279.9	13.1	4	235.2	36.4
Trimedoxim	11	334.0	29.8	5	201.4	38.2
Methoxim	21	354.7[a]	15.8	4	157.4	23.9
Obidoxim	14	419.9[a]	23.47	5	166.2	18.2

[a] Statistically significant difference from control values (saline); $p < 0.01$.

Discussion. Our previous paper (BENEŠOVÁ and HVIZDOŠOVÁ[1]) demonstrated clearly that CHE reactivators can induce an increase in glycogen concentration in liver, skeletal and heart muscle, and an increase in blood glucose level in rats. The first series of experiments confirmed again the rise of glycogen in muscle. If a rise in liver and muscle glycogen appears simultaneously with the rise of glycaemia in hungry animals, the most probable explanation is gluconeogenesis. The activation of adrenal cortex and increased release of corticosterone observed support this hypothesis. The lack of muscle glycogen rise in epinephrectomized animals may represent further evidence for this conclusion. Further findings – unchanged levels of non-esterified fatty acids and of amino acid nitrogen – need not be in contradiction, since the turnover of amino acids released from proteins and fatty acids from fats may be at such a speed that no change in these metabolites levels is detectable.

The estimation of catecholamines in adrenals, revealing a fall in concentration after the administration of CHE reactivators indicates also slight activation of the adrenal medulla. The lack of any change in the activity of liver tryptophane-pyrrolase and varying effects of CHE reactivators on the release of adrenal catecholamines (decrease of noradrenaline after methoxim and toxogonin, decrease of adrenaline after trimedoxim) is still difficult to explain.

Activation of Sustained Sympathetic Vasodilatation in Dog by Spinal Cord Stimulation[1]

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Summary. Electrical stimulation in lateral sites of the upper cervical spinal cord evoked vasodilatation after adrenergic blockade. Sympathetic fibres mediating sustained vasodilatation were shown to be separate from adrenergic sympathetic fibres since the adrenergic vasoconstrictor response in the paw evoked by vasomotor stimulation in the medulla was not reversed to vasodilatation after bretylium.

A vasodilator innervation which can be activated by sympathetic stimulation following adrenergic blockade exists in the cutaneous vasculature of the canine paw[3] and ear[4] and in the hind limb[5]. The vasodilatation brought about by stimulation of these sympathetic fibers is in part mediated by acetylcholine since the initial rapidly developing component of the response is blocked by atropine[5]. The majority of the response, however, which is of long duration is not attributable to cholinergic mediation or to release of other known transmitters[3,5] and has been termed sustained vasodilatation[5]. Because it is of importance to know whether central representation of this unique vasodilator system exists, stimulation of the lateral aspect of the upper cervical spinal cord was explored as a means of activating sustained vasodilatation. Experiments utilizing medullary vasomotor center stimulation were also conducted to try to separate physiologically the adrenergic and sustained vasodilator innervations.

Materials and methods. Experiments were carried out in 19 mongrel dogs anesthetized with 30 mg/kg of sodium pentobarbital and artificially ventilated after administration of 0.25 mg/kg of decamethonium bromide. Supplements of the anesthetic and neuromuscular blocker were given when necessary. With the animal in the prone position and its head placed in a holder, the medulla and uppermost portion of the cervical spinal cord were exposed through an incision over the cisterna magna. A bipolar concentric electrode was used to stimulate either the medulla (floor of the 4th ventricle) or the lateral spinal cord. Various sites 1–6 mm below the floor of the 4th ventricle and on the lateral aspect of the spinal cord just caudal to the medulla 1–2 mm beneath the surface were stimulated. The procedure of hindpaw perfusion which has been reported previously[4] entailed pumping blood from the femoral artery to the cranial tibial artery of the left hind paw with a Sigmamotor pump. The extracorporeal tubing encompassed 75 ml and included a 53 ml coil which delayed the passage into the paw of catecholamines released from the adrenal medulla during central elicitation of vasodilator responses. The animal was primed i.v. with 75 ml of 5% dextran to replace the blood

[1] Supported by USPHS Grant No. HL 08570 and part of thesis work by Dr. ROLEWICZ.
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