EFFECT OF SOME IONS AND BIOLOGICALLY ACTIVE SUBSTANCES ON THE ACETYLCHOLINE-BINDING CAPACITY OF THE BLOOD IN VITRO

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Potassium ions and histamine inhibit the binding of acetylcholine by red blood cells whereas calcium ions, catecholamines, and serotonin stimulate this process. Cholinolytics (oxyphenonium bromide and gangleron) increase the binding of the mediator, but only if the cholinergic receptor is not blocked as a result of treatment with the cholinolytics.

The phenomenon of binding of acetylcholine added to human blood in vitro has been described previously [2-4, 9]. This binding, one of the mechanisms of acetylcholine inactivation, may vary depending on an increase or decrease in the concentration of certain ions and biologically active substances [6, 14, 15].

The object of this investigation was to study the effect of K and Ca ions, catecholamines, histamine, and serotonin, and also of the cholinolytic drugs oxyphenonium bromide and gangleron (1,2-dimethyl-3-diethylaminopropyl p-isobutoxybenzoate hydrochloride on the acetylcholine-binding capacity of the blood.

EXPERIMENTAL METHOD

The acetylcholine concentration in a standard solution and in human blood was determined by the reaction of the isolated dorsal muscle of the leech [5]. The quantity of exogenous acetylcholine bound by blood in 24 h at 4°C was determined as the control [3, 9]. In the experiments, solutions of the test substances in concentrations indicated below were added to the blood.

EXPERIMENTAL RESULTS AND DISCUSSION

To rule out any direct action of the test substances on the preparation of the dorsal muscle of the leech, in 15 experiments an acetylcholine solution (1×10^{-8} g) containing one of the above-mentioned biologically active substances was added to it. In every case the contraction of the dorsal muscle of the leech was the same as after treatment with acetylcholine alone.

On the addition of 0.1 ml 0.02% KCl solution to 28 samples of whole blood the degree of acetylcholine binding was lowered compared with the control in every case (Fig. 1). In some cases the addition of KCl led to total inhibition of the binding power of the blood.

In 10 experiments the KCl solution was added not only to whole blood, but also to red cells washed with physiological saline. In 8 cases the acetylcholine binding by the red cells was unchanged, while in 2 cases it was slightly reduced (by 11-13%). Consequently, K ions reduce acetylcholine binding by the red cells only in the presence of plasma.

On the addition of 0.1 ml 0.02% $CaCl_2$ solution to whole blood (24 samples) the acetylcholine binding was increased on the average by 117%. On the addition of $CaCl_2$ to the washed red cells (10 samples) the acetylcholine binding was unchanged. Calcium ions thus also affect acetylcholine binding by red cells only in the presence of plasma.

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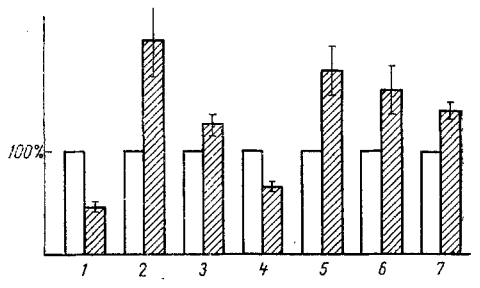

Fig. 1. Effect of ions, biologically active substances, and cholinolytics on acetylcholine binding by the blood: 1) KCl; 2) $CaCl_2$; 3) catecholamines; 4) histamine; 5) serotonin; 6) oxyphenonium bromide; 7) gangleron. Unshaded column – control, shaded – experiment. Ordinate, acetylcholine binding (in percent of control).

In those cases (10 samples of each) in which K and Ca salts were added to whole blood passed through the ion-exchange resin KI_2, or to a mixture of plasma and erythrocytes from which endogenous ions have been removed, acetylcholine binding was unchanged compared with the control. Consequently, the action of exogenous Ca and K ions on acetylcholine binding in vitro is exhibited only in the presence of endogenous ions.

Addition of adrenalin (24 samples) or noradrenalin (13 samples) to the blood in a concentration of 1×10^{-7} g led to an increase in acetylcholine binding on the average by 28%.

On the addition of 1×10^{-7} g histamine (10 samples) acetylcholine binding by the blood was reduced on the average by 26%, whereas on the addition of 1×10^{-7} g serotonin (10 samples), on the other hand, the binding was considerably increased, on the average by 83%.

The effect of cholinolytics (oxyphenonium and gangleron) in concentrations of 1×10^{-6} g was tested on blood samples from patients with gastric and duodenal ulcer treated with these drugs for 1 month (0.5-1 ml of 1.5% gangleron solution or of 0.1% oxyphenonium bromide solution subcutaneously twice or three times a day). When the blood samples were taken from the patient before the beginning or after the end of treatment (15 tests), the addition of oxyphenonium or gangleron increased acetylcholine binding by the blood by 40-60%. When the cholinolytics were added to blood samples taken on the 3rd-5th days of treatment (9 samples), these drugs did not change the binding level.

Potassium ions, with an important role in acetylcholine metabolism and cholinergic functions [1, 6], are known to participate directly in the maintenance of the morphological integrity of red cells [15]. It was shown previously [3, 9] that the acetylcholine binding power of the red cells is exhibited to the fullest only if they are morphologically intact. Endogenous K ions thus play a direct role in the binding of acetylcholine by the blood in vitro. The addition of exogenous K ions, as the results of the present experiments showed, reduces the degree of binding evidently as a result of competition between K and acetylcholine.

A decrease in the extracellular Ca concentration leads to liberation of acetylcholine from the bound form [12, 13, 15, 16]. The results of the present experiments showed that the addition of $CaCl_2$ to whole blood considerably stimulates acetylcholine binding, probably by increasing its penetration into the red cells. The opposite effect of K and Ca cations on the binding power of the red cells is exerted through certain factors connected with endogeneous ions and contained in the plasma.

The effect of adrenalin on acetylcholine metabolism has received little study although evidence in support of the synergism as well as the antagonism between these substances is sufficiently well known [1, 11]. The results of the present investigation showed that catecholamines stimulate acetylcholine binding by the bloood. Serotonin, which in certain other respects has an action similar to adrenalin on cholinergic processes and on acetylcholine metabolism [10], has an even stronger stimulant effect.

Histamine, like K ions, inhibits acetylcholine binding by the blood, and this is probably a common property of substances whose action is similar to that of acetylcholine and which induce trophotropic reactions [7, 8].

Cholinolytics (oxyphenonium and gangleron) increase the acetylcholine-binding power of the blood. However, this action is exhibited only when the binding substrate of the red cells is not blocked by cholinolytics. The ability of cholinolytics to block the binding substrate of the red cells is confirmed by earlier experiments [9] in which preincubation of blood with atropine reduced its acetylcholine-binding power.

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