Effect of Some Lectins on the Cholinergic Structures of the Frog Heart

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The effect of lectins (phytohemagglutinin - PHA, concanavalin A - ConA, *Pisum sativum* lectin - PSL, *Ricinus communis* lectin - RCL, and pokeweed mitogen - PWM) on the cardiac cholinoceptors is studied in experiments on isolated hearts of male *Rana temporaria* frogs. The test lectins in concentrations from 10^{-23} to 10^{-3} are shown to exhibit cholinomimetic properties.

Key Words: *lectins; cholinoceptors; allergic reactions*

Lectins (glycoproteins of plant origin) are increasingly attracting the attention of researchers as agents affecting the course of immune, allergic, and pseudoallergic reactions. They are also of interest for some biochemical companies as preparations used in the production of antisera. Lectins are well known to be active in skin tests. We have used the method of skin tests with PHA in allergic disorders [7]. In the present study we explored the effect of some lectins (PHA, ConA, PSL, RCL, and PWM) on the cholinergic receptors in the frog heart.

MATERIALS AND METHODS

The experiments were carried out on 62 hearts (isolated after Straub) of male *Rana temporaria* frogs weighing 50-70 g in the fall, winter, and spring (1992-1993). All test preparations were diluted with Ringer solution for coldblooded animals, the same solution being used for perfusion of the hearts. The cardiac contractions were recorded in the isometric mode using a mechanotronic force transducer. An I-10 measuring unit (supplied, like the mechanotronic transducer, with an Iskatel' device for biomedical investigations) was used as a power source and for the display and measurement of the output signal of the transducer. The cardiac contractions converted to electric signals (proportional to the biochemical parameters of the heartbeat force) were recorded with an N327-3 electronic potentiometer (ZIP Instrument Factory, Krasnodar).

We used the following lectins: PHA (Reanal), ConA, PSL, and RCL (Karlovy University, Prague), and PWM (Sigma). Lectins were used in concentrations from 10^{-23} to 10^{-3} g/ml. Acetylcholine chloride (L. Ya. Karpov Mosmedpreparaty Conglomerate, Moscow) was used in the same concentrations as the lectins. Atropine sulfate was used in concentrations from 10^{-8} to 10^{-2} g/ml. The preparations were diluted *ex tempore*. The contractions of the isolated frog heart were recorded during 3-9 h at room temperature. The volume of liquid in a cannula was monitored so as to constitute 0.2 ml. The test preparations were added to the cannula in a volume of 0.1 ml. The effect of the same volume of Ringer solution on the cardiac activity before injection of the preparations served as the pressure control. Statistical processing of the changes in the amplitudes of cardiac contractions under the influence of the test preparations was performed using a 5-point scale: 1 point - the amplitude of cardiac contractions remained unchanged after application of the preparation; 2 points - the amplitude dropped by as

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much as 25% of the initial value, this being followed by its restoration to the initial level; 3 and 4 points - the amplitude dropped 25-50% and more than 50%, respectively (in the case of 3 and 4 points a short-term, less than 30-40 sec, cardiac arrest could be observed, after which the cardiac activity was restored, but usually did not attain the initial level); 5 points - cardiac arrest lasted at least 2 min. All results were compared with the mean amplitude of cardiac contractions caused by acetylcholine (AC) in the same concentrations as the test lectins.

RESULTS

In our experiments the test lectins in concentrations from 10^{-23} to 10^{-3} g/ml were capable of exerting a negative ino- and chronotropic effect. These effects developed immediately after the injection of a particular lectin into the cannula. In high concentrations (10^{-8}-10^{-3} g/ml) the preparations could cause cardiac arrest. As a rule, the heart then spontaneously, without being washed free of the preparation, resumed its activity, and 1.5-2 min later the cardiac activity could attain the initial level. If this did not occur, the heart could easily be washed off and the cardiac activity be brought to the initial parameters. Figure 1 shows the effect of RCL and PWM on the frog heart. Evidently, even low concentrations of lectins (10^{-23}-10^{-15} g/ml) could markedly reduce the amplitude of cardiac contractions, while higher concentrations (10^{-8}-10^{-3} g/ml) led to cardiac arrest. However, after every exposure to the preparations the inhibiting effect of the lectins on the heart was rapidly eliminated (escape phenomenon). In terms of the nature of their effect on the heart, the test lectins exhibited cholinomimetic properties. However, the intensity of this effect varied among the test lectins. The effect of PHA proved to be the weakest, and of RCL the strongest. For example, an inhibiting effect of PHA in concentrations of 10^{-23}-10^{-20}, 10^{-15}-

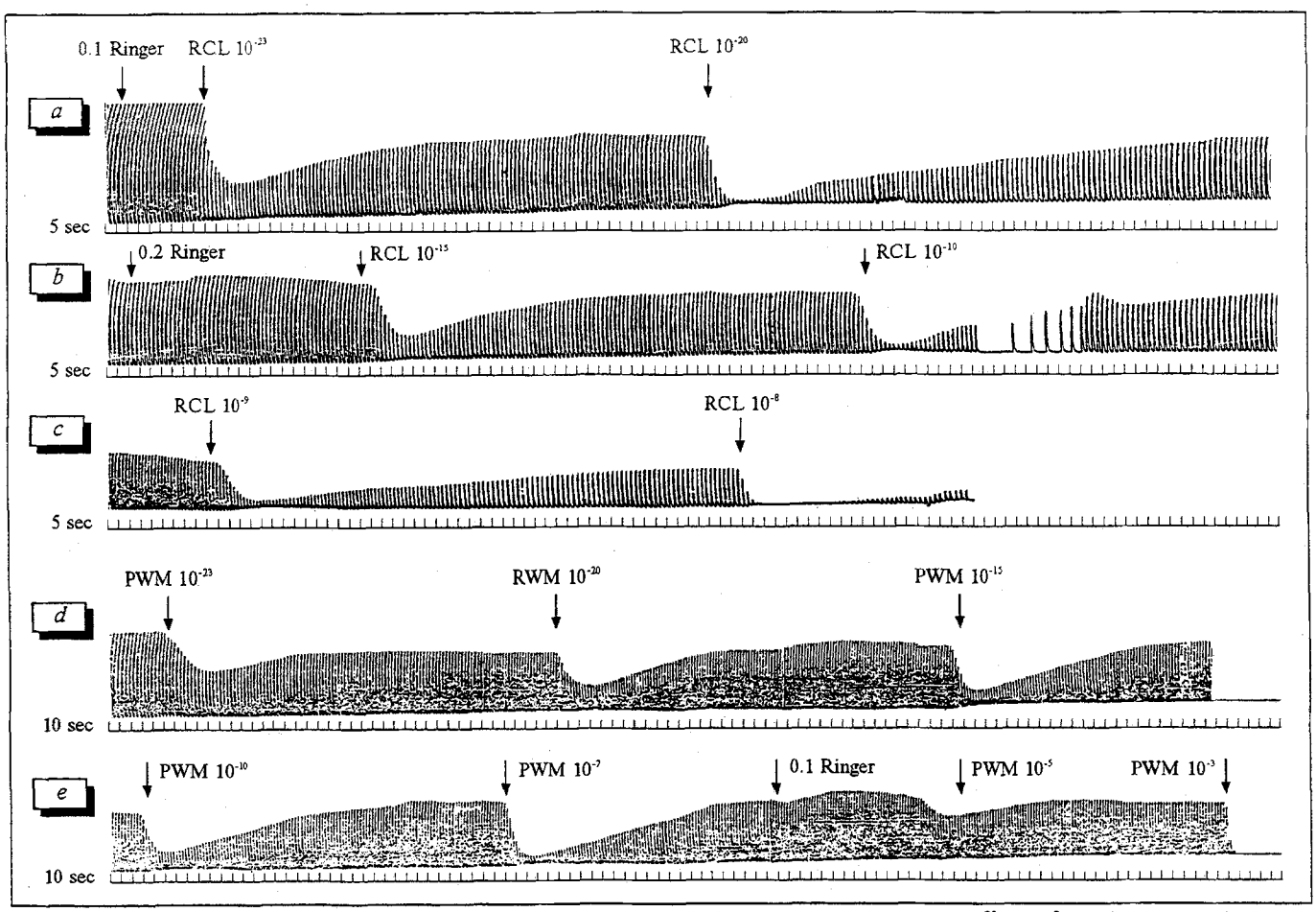

Fig. 1. Effect of lectins on the frog heart. *a*, *b*, and *c*) effect of RCL in concentrations of $10^{-23}-10^{-8}$ g/ml (time mark 5 sec); *d* and *e*) effect of PWM in concentrations of $10^{-23}-10^{-3}$ g/ml (time mark 10 sec). Here and in Fig. 3: an arrow shows the moment of injection of the preparations in a volume of 0.1 ml. Injection of 0.1–0.2 ml of Ringer solution prior to injection of the preparations serves as the control of the effect of loading on the cardiac activity. The sensitivity of the recording electronic potentiometer was 0.2 V/cm.

10^{-10}, and 10^{-5}-10^{-3} g/ml on the heart was absent in 25, 18.2, and 9.5% of cases, respectively. An inhibiting effect of RCL on the heart was absent, respectively, in 4.0, 0, and 0% of cases. As the concentration of the test lectins increased, the number of cases where there was no effect decreased. The other test lectins occupied an intermediate position (PHA<ConA<PSL<PWM<RCL).

In Fig. 2 the effects of the test lectins on the amplitude of contractions of the frog heart are compared. In the same experiments we studied the effect of AC in the same concentrations as the lectins. We see that RCL exhibited the strongest negative inotropic effect, the effects of PSL, PWM, PHA, and ConA being less marked. The inhibiting effect of lectins on the heart, as compared with that of AC, was less pronounced, but its pattern (rapid action, the presence of the escape phenomenon, stepwise changes, a ready ability to be washed off, etc.) suggested that lectins have cholinomimetic properties. This hypothesis was verified in a series of experiments on the atropine-treated frog heart. Figure 3 shows the effect of RCL (*I*) and PWM (*II*) before and after treatment of the heart with atropine in a concentration from 10^{-6}-10^{-5} g/ml; the results of 5 experiments (each of the mechanograms illustrates heart contractions of different frogs) are presented. It should be mentioned that the intensity of the effect of lectins in the same concentration could be different in different hearts. For instance, in case *I*, *a* (Fig. 3) the RCL-induced drop of the amplitude was 38.5%, in case *I*, *c* it was 79.0%, and in case *I*, *b* cardiac arrest was observed, even though in the latter two experiments the concentration of lectin was two orders of magnitude lower than in the first one. Different sensitivities of the isolated hearts to the same concentrations of preparation could also be noted in the case of other test lectins. It is also seen that atropine treatment of the heart markedly reduced the inhibiting effects of lectins (Fig. 3, *I*, *c*) or completely abrogated the negative inotropic effect caused by lectins before treatment with atropine (Fig. 3, *I*, *a* and *b*, *II*, *a* and *b*). In addition, not only was the inhibiting effect of lectins abolished, but a weak positive inotropic effect was observed. Figure 3, *II*, *a* also shows that before atropine treatment of the heart PWM in concentrations from 10^{-5} to 10^{-3} g/ml exerted a marked negative inotropic effect, which not only disappeared under the influence of atropine (10^{-5} g/ml), but also became transformed into an increase, albeit slight, of the amplitude of cardiac contractions. This is more clearly evident in Fig. 3, *II*, *b*. Basing ourselves on the notions that the cardiac receptors form a constellation system [6], we may assume that the blocking of the cholinoceptors with atropine disengages the stimulating effect of the β-adrenoceptors, which are usually inhibited by the M-cholinoceptors, and simultaneously reduces the stimulating effect on the cardiac α-adrenoceptors. A relative increase in β-adrenoceptor activity and a decrease in α-adrenoceptor activity may result in an increase of cardiac activity. In such a case lectins may enhance this effect. We also cannot rule out a direct stimulating effect of lectins on the β-adrenoceptors and their inhibiting effect on the α-adrenoceptors. The latter hypothesis demands further study.

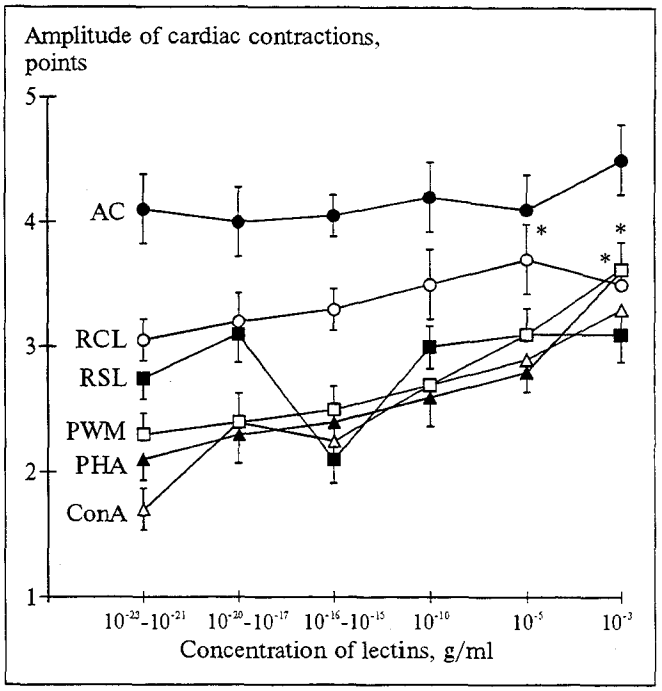

Fig. 2. Comparison of effects of test lectins on amplitude of contractions of frog heart. The mean ($M \pm m$) amplitudes of cardiac contractions caused by AC and lectins are presented. The number of observations was from 11 to 26. The effects of lectins and AC were compared for equal concentrations. Asterisks denote unreliable differences. In the other cases the differences were reliable. The confidence probability (p) was from 0.001 to 0.05.

Thus, the test lectins were able to exert a negative inotropic effect on the heart. Such an effect was most pronounced in the case of RCL and least pronounced in the case of PHA and ConA. Atropine abolished the inhibiting effect of lectins. In the atropine-treated heart preparations exposed to the test lectins a slight positive inotropic effect could be observed. We may assume that the lectins used in our experiments exerted an inhibiting effect on the heart via stimulation of the cholinoceptors. They may also exert a direct effect on the cardiac adrenoceptors, but this requires further investigation.

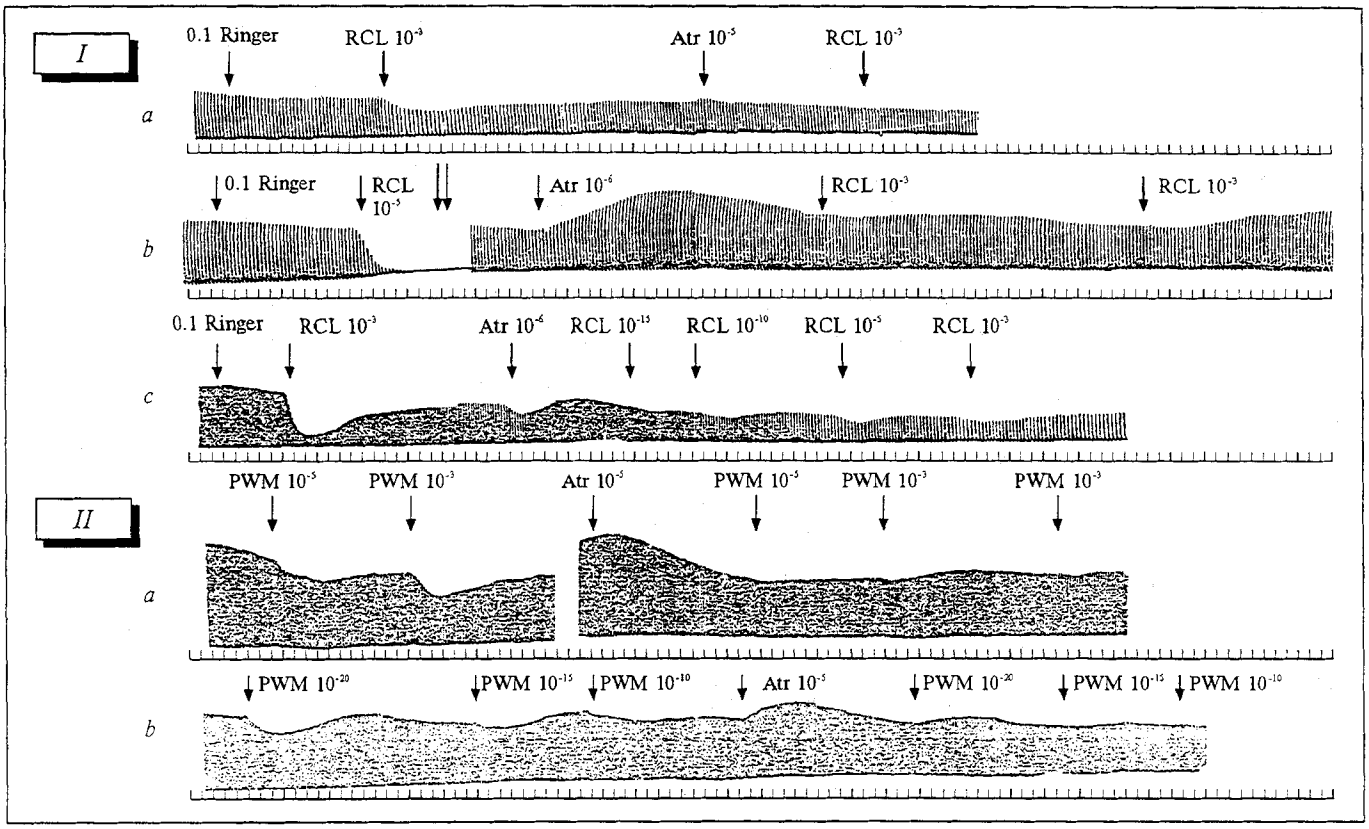

Fig. 3. Effect of lectins on the frog heart before and after its treatment with atropine. *I*: *a*, *b*, and *c*) effect of RCL in concentrations of $10^{-5}-10^{-3}$ g/ml. *II*: *a* and *b*) effect of PWM in concentrations of $10^{-20}-10^{-3}$ g/ml. One arrow shows the moment of injection of the preparations; two arrows show the moment of washing the heart with Ringer solution for coldblooded animals.

Earlier, in studies of the pathophysiological and immunopharmacological properties of lectins, we suggested that they have a cholinergic activity. This was of special interest in connection with studies of the cholinergic forms of allergic processes [1]. Lectins are known to be capable of changing the functional state of the antigen-binding receptors in murine lymphocytes, markedly reducing their ability to bind with the corresponding antigens. A hypothesis was put forward about an interaction between the lectin and immune receptors of lymphocytes, whith subsequent changes in the properties of the latter [5]. Later, it was suggested that there is a reverse interaction between the immune and lectin receptors of immunocompetent cells: changes in the functional state of the immune receptors may trigger changes in the functional state of the lectin receptors [7]. Our findings provide evidence that lectins are capable of exerting an effect upon the cardiac cholinoceptors, which broadens our view about the spectrum of activity of these preparations. Specifically, it may be assumed that lectins are capable of changing the functional state of the immune receptors, acting via the cell cholinoceptors. The relationships between the different cell receptors are now being actively studied [8-11]. It is well known that cholinomimetics are able to change the functional properties of immune receptors in lymphocytes [2-4]. The findings indicating that lectins are implicated in the cholinergic regulation of the activity of antigen-binding receptors in the cell call attention to the role of these glycoproteins in the pathogenesis of allergic reactions, and in particular cholinergic reactions.

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