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IMMUNOCHEMICAL MODELING OF LIGAND-RECEPTOR REACTIONS. COMMUNICATION V. PROPERTIES OF ANTIBODIES RAISED AGAINST THE TRIETHYLAMMONIUM DETERMINANT

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The properties of antibodies to immunogens determined by triethylammonium were studied. The immunochemical method is suggested for determining the ligands of a cholinergic receptor with a predominantly antidepolarizing type of action.

A number of studies in the last decade experimentally confirmed the reality of creating immunochemical models of ligand-receptor reactions. It was based on the possibility of obtaining antibodies whose antigen-binding center can be structurally and consequently functionally similar to the receptor binding site. For example, the immunoglobulins raised against spiroperidol (spiperone) [1], alprenolol [2], morphine [3] clonidine [4], and other physiologically active substances could sometimes recognise not only the ligand modeled by the hapten contained in an immunogen, but also its pharmacological analogs and even antipodes. Moreover, both monoclonal and polyclonal antibodies could exhibit such activity. Furthermore it seemed quite logical that anti-idiotypes raised against immunoglobulins with such a characteristic could affect biological objects like ligands of the relevant receptors and compete with them for binding to these receptors or idiotypes. The experimental results led to the conclusion that an "internal image" of the ligand is created by the an active receptor of the anti-idiotype — the polypeptide analog of a chemical physiologically active substance. This indirectly confirmed a certain adequacy of the "antibody" model, with which the image of the ligand natural to a receptor was created.

In our previous studies [5], we reported on the preparation of antisera by means of which we developed an enzyme immunoassay (EIA) making it possible to selectively determine cholinergic substances. Subsequently, the multipotent universal nature of recognition of agonists and antagonists of the nicotine and muscarine receptors could be altered in the course of the immunosorption procedure. This made it possible either to separate antibodies with abilities to recognize cholinergic ligands to a considerable extent similar to those for an H-cholinergic receptor in an activated state, or to deprive this antiserum of the ability to react with anticholinergic agents. Detailed study of these antibodies made it possible to modify the enzyme immunoassay of the agonists of the acetylcholine receptor. This made possible a more adequate approach to assaying the activity and metabolism in an organism of choline esters of dicarboxylic acids, which are substrates of cholinesterases [6]. Moreover, in a system combining use of antibodies and cholinesterase, the possibility was shown in principle of creating a screening model for selecting chemical compounds with anticholinesterase activity. Next the presence in the antibody pool of immunoglobulins with catalytic properties was proved in the experiments. This confirmed that the immunochemical approach to modeling any type of specific reaction was realistic.

Also, in further studies of the reaction of antibodies modeling the recognition by an H-cholinergic receptor with enantiomers of 3-quinuclidine derivatives, substances with a pronounced cholinomimetic activity were discovered among the latter. Stereospecificity of recognition in the series of compounds of the given group was also established [7]. From our viewpoint, this was the most significant proof of the receptor-like activity of the antibodies being studied.

Finally, the binding agent obtained as a result by immunization of the quaternary ammonium base could also recognize a tertiary amine, the classical H-cholinomimetic nicotine, and distinguish between trimethylammonium cholinergic agents (agonists of the cholinergic receptor), and triethylammonium cholinergic agents that are antidepolarizers [5]. It was exactly this fact that was the premise for the present studies aimed at establishing the properties of antibodies to immunogens determined by triethylammonium and comparing these properties

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o a solution of 0.31 g (0.00088 mole) of
oxyethyltriethylammonium bromine hy-
ixture of 5 ml of pyridine and 2.5 ml of
d the precipitated crystals, washed them
ıer, and crystallized them from ethanol.
g (68%) of 4-aminophenylhydroxyethyl-
bromide hemisuccinate, melting at 206°C.
nposition of the product was confirmed

onjugates. Conjugates of bovine albumin
ulin with 4-aminophenylhydroxyethyltri-
omide were obtained in an azocoupling re-
juagates with the hemisuccinate of this hap-
with the aid of a water-soluble carbodiimide

ɔn of antisera, isolation of antibodies, and
EIA were described in detail previously [5].
re the efficacy of various substances used as
ody binding with a solid phase, we used the
tration that ensured a 50% level of inhibition
antibodies in comparison with the control

) DISCUSSION

: sera of two groups of animals immunized
antigens based on 4-aminophenylhydroxy-
ıonium or its hemisuccinate made it possible
ıidirectional nature of the reaction of the anti-
era with triethyl and trimethylammonium cho-
In both cases, the competitiveness of the sub-
ıdied with respect to the solid-phase 4-amino-
:ythylammonium successively diminished in
methylenebis(triethylammonium), tetraethyl-
:camethylenebis(trimethylammonium), tetra-
um (Fig. 1, *IIa*).
shed feature of recognition by the antibodies
in the given group differed from the nature of
th these compounds of antibodies to hapten in-
ıylammonium. The reason is that for the latter
:amethylenebis(triethylammonium) had a more
:tivity in comparison with tetramethylammoni-
ı). Furthermore, studying the influence of
'lcholine, and butyrylcholine as inhibitors of
e antibodies raised against triethylammonium
ıame regularities in the efficacy of these com-
the immunoglobulins raised against the triethyl-
eterminant [5]: namely, it was greater for buty-
ın for acetylcholine, and even more pronounced
ne. However, while the previously described dif-
veen the concentrations of choline and butyryl-
ıg to an equal effect reached a factor of 20, in the
/ when using antibodies raised against a hapten
r" ethyl radicals at the quaternary nitrogen atom,
e was only a factor of 4.5.

Similar results were also obtained in a comparative study of the dicholine esters of adipic, suberic, and sebacic acids. As follows from previous results [6], the differences between the equally effective concentrations of trimethylammonium and the esters of dicarboxylic acids under study could differ up to four orders of magnitude (IC_{50}, 6×10^{-4} and 2×10^{-7}, 1.5×10^{-7}, 6×10^{-8} mmole, respectively), while for antibodies to triethylammonium, the maximum differences with respect to this parameter reached a factor of 20 (5×10^{-4} and 5×10^{-5}, 3.2×10^{-5}, 2.45×10^{-5} mmole, respectively).

Of definite interest from the above discussion, was the study of the reaction of antisera containing antibodies raised against an immunogen determined by the triethylammonium group with some of the most active (as we established earlier [7]), derivatives of 3-quinuclidine. In the series of polymethylenebis(quaternates) of quinuclidine, the activity of the compounds of this group (expressed as their IC_{50}) increased successively with an increase in the number of polymethylene units; and for hexamethylene-, octamethylene-, and decamethylenebis[S-(–)-3-chloroquinuclidine-chloride], the IC_{50} was 1.2×10^{-6}, 4.0×10^{-7}, and 2.2×10^{-7} mmole in the test sample, respectively. Consequently, the range of the differences between the equally effective concentrations of these compounds was about a factor of 5.5, while for antibodies raised against trimethylammonium the differences exceeded three orders of magnitude [7]. Moreover, the antibodies to the triethylammonium determinant could not distinguish between the enantiomers of 3-chloronuclidine hexadecylbromide. In contrast, the antibodies modeling recognition by the H-cholinergic receptor of nicotine agonists (all the way up to muscle relaxants with a depolarizing type of action) demonstrated stereospecific recognition in the given series.

On the contrary, the more than moderate reaction of antibodies recognizing agonists of the nicotine cholinergic receptor with curare-like compounds, derivatives of cyanuric acid [10] and some Gangleron (ganglefene), pachycarpine, pentamin (azamethonium bromide), hexamethonium] when using antibodies raised against hapten with triethylammonium radicals led to results suggesting considerable activity for these compounds. For example, the overwhelming majority of the studied cyanurates (IC_{50} from 1.5 to 2.5×10^{-7} mmole) whose synthesis and physiological activity were described earlier [11] exhibited affinity for antibodies close to that for decamethylenebis(triethylammonium), the most active of the substances we studied (IC_{50} 1.0×10^{-7} mmole in the sample).

In our previous studies [5, 10], we repeatedly showed the possibility of controlling the specificity of recognition of cholinergic agents in the coure of EIA. For instance, when using the relevant immunosorbents, physical separation of the antibodies responsible for binding with a group of compounds and also for the removal from the serum pool of antibodies hindering analysis is feasible. Next, the variety of immunochemical analysis we used made possible controlled manipulation of the properties of a specific binding agent by varying the structure of the solid-phase hapten competing with the free ligand being analyzed for the antibody binding site.

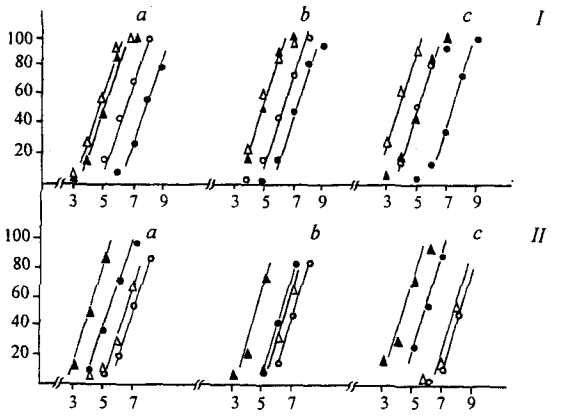

Fig. 1. Specificity of antibodies raised against trimethylammonium (*I*) and triethylammonium (*II*) determinants in reaction with three-phase homoleogous (a), heterologous (b) haptens and with a homoleogous hapten after immunosorption purification (c). Abscissa) The amount of substances, mmole; ordinate) the percent of antibody binding. The ligands are: ○) DEA — decamethylenebis(triethylammonium bromide); ●) DMA — decamethylenebis(trimethylammonium) bromide; ▲) TMA — tetramethylammonium bromide; and Δ) TEA — tetraethylammonium bromide.

In the present work, we also studied the possibility of controlling the specificity of both immunosorption and functional analysis using the example of the two types of antibodies being compared herein. The results of the given experiments are presented in Fig. 1 and in Table 1. They reveal that the use in EIA of solid-phase heterologous hapten (i.e., differing from that used to prepare the given antibodies) in both cases increased the competitiveness of the homoleogous ligands, in other words ligands with a similar structure. It can be seen that replacement of the trimethylammonium determinant on the solid

TABLE 1. Quantitative Parameters of Reaction of Antibodies with Solid-Phase Hapten (IC_{50}, mmole in sample)

Reaction variant	Ligand			
	DMA	DEA	TMA	TEA
Ia	1.3×10^{-8} (1.0)	4.5×10^{-7} (0.29)	7.9×10^{-6} (0.0016)	1.8×10^{-5} (0.0007)
Ib	7.9×10^{-8} (1.0)	5.6×10^{-7} (0.14)	1.4×10^{-5} (0.0056)	1.4×10^{-5} (0.0056)
Ic	4.5×10^{-8} (1.0)	1.0×10^{-5} (0.0045)	1.0×10^{-5} (0.0045)	2.0×10^{-4} (0.0002)
IIa	4.5×10^{-6} (0.029)	1.3×10^{-7} (1.0)	1.1×10^{-4} (0.0012)	3.6×10^{-7} (0.36)
IIb	6.3×10^{-7} (0.14)	8.9×10^{-8} (1.0)	3.2×10^{-5} (0.0029)	2.8×10^{-7} (0.32)
IIc	1.7×10^{-6} (0.0046)	7.9×10^{-9} (1.0)	4.5×10^{-5} (0.0002)	2.1×10^{-8} (0.38)

Note. The relative inhibitory index is given in parenthesis. Variants of the interaction and abbrevations for the ligands are presented in Fig. 1.

phase for antibodies raised against trimethylammonium with the triethylammonium determinant (Fig. 1, *Ib*) resulted in a fivefold relative growth in the recognition of decamethylenebis(triethylammonium) and in an almost eightfold increase in recognition of tetraethylammonium in comparison with decamethylenebis(triethylammonium). A similar effect, but in the opposite situation, can be seen for antibodies to the triethylammonium determinant (Fig. 1, *IIb*).

Conversely, the use in EIA of an antiserum from which the antibodies reacting with the heterologous hapten were removed by immunoaffinity chromatography unambiguously led to weakening of the recognition of the structures not represented in the immunogen that induced the synthesis of antibodies (Fig. 1, *Ic*, *IIc*). It was thus shown that such processing of a binding agent can alter its reaction parameters in the direction of a many-fold increase in the specificity for analysis of cholinergic compounds with a homoleogous structure with respect to the antibodies used in the EIA.

The results of the present experiments indicate that we have obtained an analytical tool that can selectively reveal ligands of a nicotine cholinergic receptor with a chiefly antidepolarizing type of action.

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