SENSITIZING AND DESENSITIZING INFLUENCE OF ATROPINE, BETE*, AND AMYSYL ON THE SPASMOLYTIC ACTIVITY OF PENTAPHENE AND TROPACINE

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In a previous study, we investigated the combined action of a number of cholinolytic preparations (complex esters of tropine or dialkylaminoethanol with various aromatic acids) on experimentally induced hyperkinesis of central origin. These observations were made with the use of an arecoline model of hyperkineses in mice, and we found that the combined application of certain cholinolytic substances produced varying degrees of mutual potentiation, additivity, relative antagonism, or synergo-antagonism [1]. As an extension of certain observations [2], it was also shown that in the combined application of derivatives of an aromatic acid and of an aromatic oxyacid, the potentiating type of synergism did not always occur.

The present investigation has been made to extend the study of certain combinations of the same series of cholinolytic preparations, and to determine their influence on hyperkinesis in mice induced by the injection of nicotine. We have tried at the same time to determine in what way these combinations of substances produce their arecoline and nicotine actions. As previously, we have studied how the action of the combination is influenced by the simultaneous application of aromatic acid derivatives and derivatives of aromatic oxyacids. The analysis of the action of the preparations was made graphically [5].

EXPERIMENTAL METHOD

The experiments were carried out on white male mice weighing from 20 to 24 g. As a basis for the investigation, the mice received into the back a subcutaneous injection of 7 mg/kg nicotine given as a 0.1% solution. With this method of injection and the doses indicated, all the animals developed a characteristic tremor, or, in rare cases, convulsions. The solution of nicotine was prepared immediately before use.

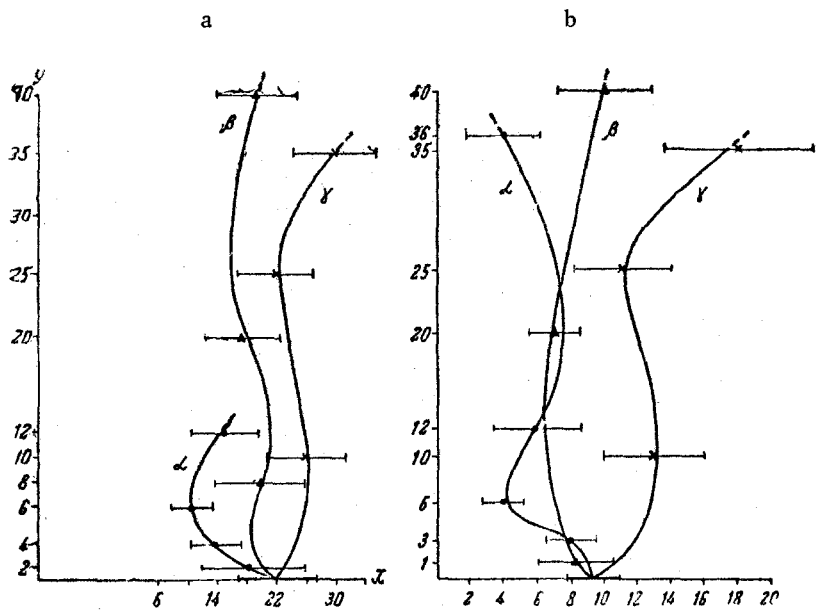
Atropine sulfate, scopolamine bromhydrate, tropacine, BETE, pentaphene, and amysyl chlorhydrate were injected intraperitoneally; the actual volume of fluid given did not exceed 0.6 ml. Each combination consisted of two substances. The analysis and evaluation of the action of these substances was made graphically [5, 7].

Because the combinations of substances studied included some (atropine, BETE, and amysyl) which have little influence on nicotine hyperkinesis, to analyze and evaluate the action of the combinations, we used a variation of Loewe's method which enables the mutual influence of oppositely acting substances to be determined [8]. For the component of the combination which was effective in 100% of the animals (let us call it component B), we determined the dose which prevented tremor in 50% of the cases (ED^{B}_{50})**, and we plotted this dose and its confidence limits as abscissa. Then in subsequent experiments we again determined ED^{B}_{50}, but under conditions when the animals had previously received the second ineffectual component of the mixture (component A)***, the component which failed to prevent tremor from occurring in 100% of the animals.

*$\overline{\text{BETE}}$—chlorhydrate of the tropine ester of benzylic acid [3].

**The ED_{50} was calculated by the probit method of Miller and Tainter [9] for $P = 0.05$, and $t \leq 1.96$.

***In all the experiments, component A was injected 15 minutes before the standard dose of 7 mg/kg nicotine. Component B was injected 10 minutes before the nicotine.



Influence of various doses of atropine, BETE, and amysyl on the spasmolytic effects of (a) pentaphene and (b) tropacine on nicotinic hyperkinesis in mice. Ordinate—doses of atropine, BETE, amysyl (mg/kg); abscissa—doses of pentaphene (a) and tropacine (b). Scale on the abscissa is arbitrary. α — mean value curve of a combination of atropine + pentaphene (a), and BETE + tropacine (b); β — mean value curve of a combination of BETE + pentaphene (a), and BETE + tropacine (b); γ — mean value curve of a combination of amysyl + pentaphene (a), and amysyl + tropacine (b).

Component A was progressively increased from one experiment to the next, and in each case the ED^{B}_{50} was determined. The values of the ED^{B}_{50} so found were plotted on a graph, and through them the curve was drawn showing the effect of component A on the selected effect of component B (see figure, α, β, γ).

The nature of the action of both components when given together was evaluated as follows. If the values of the ED^{B+A}_{50} and the curve drawn through it on the graph are significantly inclined towards the ordinate, the action of component A on the effect of component B is said to be "sensitizing." If there is a significant deviation away from the ordinate, to the right, component A is considered to have a "desnsitizing" effect on component B. Finally, if the deviation of the values of the ED^{B+A}_{50} from the ED^{B}_{50} was not significant, it was assumed that component A had no influence on the particular effect of component B (the drawing shows such a curve for pentaphene (a) and for tropacine (b) when used together with atropine, BETE, and amysyl).

EXPERIMENTAL RESULTS

Table 1 shows the results of experiments in which the ED_{50} of pentaphene and tropacine was determined on mice in which hyperkinesis had been induced. As a comparison we give also the ED_{50} of the same preparations when the two were given, together with various doses of atropine, BETE, and amysyl. These results are shown graphically in the figure, where the ED_{50} and the confidence limits for (a) pentaphene and (b) tropacine are plotted on the abscissa. The remaining points on both graphs represent the values of the ED_{50} of tropacine and pentaphene, in the case when both preparations were used together with various doses of atropine, BETE, and amysyl.

The combined application of pentaphene with atropine, BETE, and amysyl causes a change in the ED_{50} of pentaphene. The change in the effectiveness of the latter shows up differently, and depends on which of the three preparations was mixed with it. Thus, when pentaphene was given in combination with atropine, the "sensitizing" influence of the latter was brought out very strongly, and was best developed with a dose of 6 mg/kg. With increase in the dose of atropine, its "sensitizing" action with respect to the "antinicotinic" effect of pentaphene was reduced (curve α deviates somewhat to the right, away from the ordinate (see figure, a)).

In the combination of pentaphene with BETE and amysyl, in the doses used, the latter two substances do not influence the effectiveness of pentaphene (the difference of the value of the ED_{50} of pentaphene + amysyl, or

TABLE 1. Influence of Atropine, BETE, and Amysyl on the Effectiveness of Pentaphene and Tropacine in Combating Nicotine Poison in Mice (Nicotine Hyperkinesis)

Component A	Dose of component A (mg/kg)	Component B	ED^{B}_{50} and confidence limits (mg/kg)	Observed effect
Atropine	–	Pentaphene	22.0±5.25*	Component A sensitizes to component B
	3		14.0±3.6	
	6		11.5±2.94	
	12		15.0±4.3	
BETE	–	"	22.0±5.25	Component A does not influence the effect of component B
	1		19±7.35	
	8		20±5.9	
	20		18±5.01	
	40		20±5.3	
Amysyl	–	"	22.0±5.25	Component A does not influence the effect of component B
	10		26±5.3	
	25		22±5.1	
	35		30±5.6	
Atropine	–	Tropacine	9.4±1.56	Component A sensitizes to component B
	3		8.0±1.65	
	6		4.0±1.25	
	12		6.0±2.74	
	36		4.8±2.28	
BETE	–	"	9.4±1.56	Component A does not influence the effect of component B
	1		8.6±2.16	
	20		7.8±1.61	
	40		10.5±2.8	
Amysyl	–	"	9.4±1.56	Component A desensitizes to component B
	10		13±3.01	
	25		11±2.89	
	35		18±4.64	

*ED_{50} of component B in the absence of component A.

TABLE 2. Effects Observed in the Combined Application of a Number of Cholinolytic Substances Given to Mice Poisoned with Arecoline and Nicotine (Arecoline and Nicotine Hyperkinesis in Mice)

Combination	Observed effect	
	Action with arecoline*	Action with nicotine
Atropine + pentaphene	Synergism, potentiating type	Sensitization
Scopolamine + pentaphene†	" " "	"
Atropine + tropacine	" " "	"
BETE + pentaphene	Additivity	No effect
Amysyl + pentaphene	"	" "
BETE + tropacine	"	" "
Amysyl + tropacine	Synergism, potentiating type	Desensitization
Atropine + scopolamine‡	Additivity	–
BETE + scopolamine‡	Synergism, potentiating type	–
BETE + amysyl‡	Additivity	–

*Our own data are given [1].

†Published data given [2].

‡Both components are almost ineffectual in combating the effects of nicotine.

pentaphene + BETE and that of pentaphene alone, were not statistically significant).

An examination of Table 1 and the figure (b), giving the results of the combined application of tropacine with atropine, BETE, and amysyl, shows that in this case, too, the effect of the three substances concerned on the antinicotinic effect of tropacine is manifested in various ways. Consider curve α_1; it represents the effectiveness of tropacine given together with atropine, and in certain parts the graph approaches the ordinate. This approach indicates that 6 or 36 mg/kg of atropine increases the effectiveness of tropacine. With regard to the influence of BETE, a dose of 16 mg/kg somewhat increases the effectiveness of tropacine, but this result was not statistically significant.

On the other hand, amysyl given as a large dose of 35 mg/kg together with tropacine enhanced the effectiveness of the latter, as indicated by the ED_{50}, i.e., it reduced the antinicotinic activity of tropacine. A comparison of the influence of atropine, BETE, and amysyl on the effects of tropacine and pentaphene indicates that in all cases atropine had a "sensitizing" effect; amysyl given together with tropacine reduced the "antinicotinic" action of the latter, but has no effect on that of pentaphene, whereas BETE is always "indifferent."

The "sensitizing" action of atropine on the "antinicotinic" effects of pentaphene were observed in experiments on four rabbits. For each animal, we first found the minimum dose of nicotine which always caused a marked hyperkinesis. An injection of 6 mg/kg atropine 15 minutes before the nicotine influenced neither the nature nor the development of the normally occurring convulsions (nicotine hyperkinesis). The injection of the same amount of atropine followed by pentaphene led in all cases to a considerable reduction of the minimum effective dose of pentaphene. The effectiveness of pentaphene was increased almost 50%.*

In addition to the combinations shown in Table 1, to determine the possible manifestation of the "coalitive action" [8] of the preparations in nicotine hyperkinesis, we made an investigation of the combined action of such pairs of substances as atropine and scopolamine, BETE and amysyl, BETE and scopolamine. However, the combinations of these compounds, even in considerable doses, did not lead to the development of any noticeable "coalitive" effect. The results obtained on the "sensitizing" action of atropine when given together with pentaphene, to a certain extent confirm the observations of N. A. Kharauzov [2], who observed a similar "sensitizing" action of scopolamine on the antinicotinic effect of pentaphene. It is highly probable that the effect we have found for atropine is associated with the fact that in many cases [4] it is still able to exert an antinicotinic spasmolytic action.

A comparison was made of the results of the present and previous investigation (experiments with arecoline hyperkinesis in mice); it showed that in most of the combinations studied (Table 2) in which there was a synergism of a potentiating type in arecoline hyperkinesis, with nicotine hyperkinesis there were sensitizing effects. Substances which in the first case acted additively, showed no effect on each other in the second. However, this result was not invariable: with a combined application of amysyl and tropacine in arecoline hyperkinesis their effects were mutually potentiating, whereas in nicotine hyperkinesis amysyl exerted a "desensitizing" action on the effect of tropacine.

In evaluating the effects of the combinations from the point of view of the chemical structure of the two components (combined application of derivatives of aromatic acids and aromatic oxyacids), it must be emphasized that just as with arecoline hyperkinesis, in the combined action of cholinolytic substances on nicotine hyperkinesis, no definite significance of a combination of derivatives of aromatic acids with those of aromatic oxyacids could be demonstrated.

SUMMARY

A study was made of the combined effects of a number of cholinolytic agents, comprising: atropine, amysyl, BETE (chlorhydrate of the tropine ester of benzylic acid), pentaphene, tropacine, and scopolamine. Substances were given in pairs. Nicotine hyperkinesis in mice was used as an experimental model. The action of the substances was evaluated by Loewe's method (1928). The results showed that atropine and pentaphene given together caused a rise in the effectiveness of the latter in preventing tremor from nicotine (atropine "sensitizes" to pentaphene). No such effect was observed when pentaphene was given together with such preparations as BETE and amysyl. Atropine potentiated tropacine; conversely tropacine reduced the effect of amysyl ("desensitization"). Under these conditions, BETE did not significantly influence tropacine action. The results obtained indicate the very diverse actions of these drugs in producing "sensitiziation," "desensitization," etc. when given together to animals in which nicotine hyperkinesis had been induced.

*The minimum effective dose of pentaphene was determined for each normal individual. In two cases it was 2 mg/kg and in two 2.5 mg/kg.

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