THE ACTION OF SMOOTH MUSCLE STIMULATORS STUDIED BY CORRELATION ANALYSIS

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Correlation between the maximal effects of acetylcholine and other cholinomimetics and also of serotonin, barium chloride, and a depolarizing solution on an isolated strip of rat stomach was studied. The effect of acetylcholine correlates closely with the effects of cholinomimetics, less closely with the effects of barium chloride and depolarizing solution, and less closely still with the effects of serotonin. It is suggested that the degree of correlation be used to analyze the points of action of drugs and the paths linking receptor stimulation with the observed effect. The point of convergence of the linking paths for the last three stimulators was shown to precede the convergence of their common path with the linking path of the effects of cholinomimetics.

KEY WORDS: smooth muscles; cholinomimetics; correlation analysis.

To analyze the points of action of drugs methods of pharmacological analysis based on the use of antagonists with competitive action are nowadays being used [2]. Dispersion of the effects of pharmacological agents, caused by variability of the properties of the test object, enables statistical methods to be used for this same purpose [4]. It is evident that if the points of action of drugs (bioreceptors) and the paths for production of their effects (linking paths) coincide, correlation will be observed between the magnitudes of their effects with a coefficient close to unity, but otherwise correlation must be absent. If significant correlation is present but with a coefficient substantially below unity, the presence of common linking stages can be postulated. In this case, the higher the coefficient of correlation, the closer the point of convergence of the linking mechanisms to the bioreceptors.

The object of this investigation was to determine the specific character of the points of application of drugs causing contraction of fragments of rat stomach. Methods of correlation analysis were used for this purpose.

EXPERIMENTAL METHOD

Isolated strips of rat stomach were used for experiments by the usual method [9]. Contractions were recorded isotonically with a load of 2 g. The experiments of series I consisted of measurement of the absolute contractions during exposure to maximal concentrations [5] of acetylcholine and of one of five cholinomimetics (carbachol, furmethide, arecoline, aceclidine, and pilocarpine) on the same strip of smooth muscle. In the experiments of series II the contractions were measured during exposure of the same strip to acetylcholine (10^{-3} g/ml), barium chloride (10^{-3} g/ml), an isotonic solution of potassium chloride (depolarizing solution), and serotonin (10^{-3} g/ml). The order of exposure to the various substances in this series followed all possible permutations in order to reduce the mutual effect of one substance on the rest (24 experiments altogether). Between exposures to the different substances the Tyrode's solution in the bath was changed four times in the course of 5-7 min in both series of experiments. In all the experiments of series II the length and weight of the strip were measured. To analyze the data, coefficients of correlation were calculated between all possible pairs of values of contractions, expressed in absolute terms. In

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TABLE 1. Coefficients of Correlation between Maximal Effects of Cholinomimetics

Pairs of substances tested		Number of experiments	Coefficient of correctation
Acetylchloine – carbachol	(10^{-4} g/ml)	4	0,95
" — furmethide	(10^{-5} g/ml)	4	0,91
" aceclidine	(10^{-4} g/ml)	4	0,91
" arecoline	(10^{-5} g/ml)	5	0,98
" pilocarpine	(10^{-5} g/ml)	5	0,90

Note. $P \leq 0.05$ for $r \geq 0.90$ ($n = 4$) and $r \geq 0.80$ ($n = 5$).

TABLE 2. Correlation Matrix between Values of Contractions Evoked by Acetylcholine (A), Barium Chloride (B), Depolarizing Solution (D), Serotonin (S), and Also with Length (L) and Weight (W) of Smooth-Muscle Preparation

	A	B	D	S	W	L
A	1	0,66	0,59	0,24	0,49	0,62
B	0,66	1	0,83	0,48	0,20	0,34
D	0,59	0,83	1	0,58	0,25	0,20
S	0,24	0,48	0,58	1	0,097	0,07
W	0,49	0,20	0,25	0,097	1	—0,02
	0,62	0,34	0,20	0,07	—0,02	1

Note. Coefficient of correlation does not differ significantly from 1.0 ($P \leq 0.05$ for $r \geq 0.665$ and is significantly below 1.0 but over 0.0 for $0.665 > r > 0.405$.

the experiments of series III the relative values of the contractions arising under the influence of the smooth-muscle cell stimulators in the concentrations given above and in the presence of manganese chloride (10^{-3} g/ml) were investigated. For this purpose the $MnCl_2$ was added to the Tyrode solution (the bicarbonate and phosphate were excluded), and during constant exposure to this solution the contractions evoked by serotonin, depolarizing solution, barium chloride, and acetylcholine (in that order) were recorded.

EXPERIMENTAL RESULTS AND DISCUSSION

Coefficients of correlation between the effects of acetylcholine and the other cholinomimetics are given in Table 1. Clearly the coefficient of correlation did not fall below 0,90, indicating a common point of application for the action of the drugs (the muscarinic cholinoceptor), in agreement with results obtained by traditional methods [1].

The results of the experiments of series II are given in Table 2 as a correlation matrix. Analysis of the matrix shows that the substances used can be divided into three groups: 1) barium chloride and the depolarizing solution, which evoked contractions correlating strongly with each other, less strongly with the effects of acetylcholine and serotonin, but not correlating at all with the length and weight of the preparation; 2) acetylcholine, the effect of which correlates significantly with contractions evoked by the substances of the previous group and also with the weight and length of the smooth-muscle preparation; 3) serotonin, whose effects correlate with contractions evoked by the substances of the first group but not with the acetylcholine contractions or with the weight and length of the preparation.

These results indicate differences in the points of action and linking paths for the effects of the substances belonging to these three groups. It can accordingly be concluded that specific cholinergic and serotoninergic receptors exist. The method as proposed can therefore be used for the classification, differentiation, and identification of receptors. Its advantages lie in the less work entailed and its freedom from the presence of specific antagonists. A similar approach, but in an implicitly qualitative form, has been used previously (see [2], pp. 154-155).

The splitting of the correlation matrix into three groups can also provide a basis for estimation of the structure of the paths linking the initial stimulus evoked by the action of the substance with the effect observed. Since the coefficient of correlation between the effects of acetylcholine and serotonin was significantly lower than the coefficient of correlation between the effects of serotonin and barium chloride or serotonin and the depolarizing solution (Table 2), the point of convergence of the linking path in the mechanism of the acetylcholine effect with the common path probably lies more distally than the point of convergence of the linking path for the serotonin effect. The hypothetical linking structure appears to be as follows:

An attempt was made to prove this hypothesis by the classical method of pharmacological blockade. According to existing views of the mechanism of action of serotonin and depolarizing solution an important role is ascribed to the entry of external calcium as the cause of contraction [7, 10]. The probable mechanism of action of barium is direct activation of actomyosin [8]; the passage of barium through the membrane is determined by its calcium permeability. Consequently, the calcium permeability of the membrane may be a common factor and may determine the point of convergence of the linking paths for serotonin, barium,

TABLE 3. Effect of Manganese Chloride on Maximal Contractions Evoked by Acetylcholine (A), Barium Chloride (B), Depolarizing Solution (D), and Serotonin (S)

Medium	Magnitude of contractions (in %)				
	relative to control	relative to acetylcholine effect			
	A	A	B	D	S
Tyrode solution	100	100	78 (24)	66 (24)	45 (24)
Tyrode solution and $MnCl_2$ (10^{-3})	84 (6)	100	4 (6)	12 (6)	1 (6)

Note. Number of experiments in parentheses. Differences between control and experiment significant when $P < 0.05$.

and depolarizing solution. To verify this hypothesis the effect of manganese chloride was studied as a substance blocking calcium permeability [6] on the magnitude of contractions evoked by acetylcholine, barium chloride, depolarizing solution, and serotonin.

The results of these experiments, given in Table 3, show that manganese selectivity depresses the effects of barium chloride, depolarizing solution, and serotonin, but causes only very slight changes in acetylcholine contractions. These results confirm the conclusion drawn from analysis of the correlation matrix, namely earlier convergence of the linking paths for the effects of barium chloride, depolarizing solution, and serotonin. By analysis of the correlation matrix definite conclusions can thus be drawn regarding the structure of the linking paths. The use of more powerful methods of analysis (factor analysis, for example [3]) could perhaps increase the resolving power of this method.

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