CONNECTION BETWEEN INTERNAL ACTIVITY AND MAXIMAL EFFECT

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Experiments on strips of rat stomach showed that the maximal effects of the muscarine-like cholinomimetics investigated, dissolved in Tyrode solution, are practically identical, but in a depolarizing solution they differ substantially. It is concluded that the ability of substances to activate a receptor cannot be described by a single parameter (the internal activity or effectiveness) without analysis of the pathways of pharmacomechanical coupling.

The molecular theory of drug action postulates that a pharmacological effect is a function of the number of receptors activated by the drug and the degree of their activation [3, 4, 10]. The ability of agonists (i.e., stimulators) to activate a receptor has been called their "internal activity" [4] or "effectiveness" [10]. This property is an expression of the physicochemical characteristics of the drug, i.e., it is determined by its chemical structure. Hence, it follows that, when all receptors are occupied (and no reserve of receptors is available) the relative maximal effect of the drug is determined entirely by its internal activity and can be used as a measure of it.

The conditional and erroneous nature of the definition of internal activity based on the magnitude of the maximal effect has previously been described [3]. The present investigation has shown that the maximal effects of certain agonists acting on the same receptors may vary differently with a change of the experimental conditions, although the internal activity, reflecting the physicochemical properties of the drug, remains constant.

EXPERIMENTAL METHOD

Experiments were carried out on strips of smooth muscle from the fundus of the rat stomach, placed in a thermostatically controlled (37°C) bath for isolated organs. The bath was filled with Tyrode solution or with depolarizing solution (isotonic K_2SO_4 solution + 1g/liter glucose), aerated with oxygen. Isotonic contractions of the strips were recorded during stretching of the specimen with a load of 2 g. The stimulants used were acetylcholine, carbachol, furmethide, arecoline, aceclidin, and pilocarpine. Their effect was studied within the concentration range of 10^{-9}-10^{-3} g/ml by plotting cumulative curves (each successive concentration was ten times higher than the one before). The duration of exposure of the specimen to each concentration was 2-3 min. The relative magnitude of the maximal effect of the drugs tested was assessed by comparison with the effect of acetylcholine (10^{-3} g/ml).

To assess the generality of the stimulated receptor, the value of pA_{10} for atropine was determined by Schild's method [7, 8]. The coefficient of correlation between the various properties of the drugs was determined by Spearman's method [1], and the other types of statistical evaluations were made by the usual methods [2]. Each series of experiments was carried out on 4-6 strips of stomach.

EXPERIMENTAL RESULTS AND DISCUSSION

The results given in Table 1 show that the magnitudes of the maximal effects of the tested drugs in Tyrode solution were virtually identical, whereas in depolarizing solutions they differed.

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TABLE 1. Properties of Muscarine-like Cholinomimetics Acting on Smooth Muscles of the Stomach under Different Experimental Conditions

M-cholinomimetic	Magnitude of maximal contraction, relative to acetylcholine maximum			Gradients of log concentration – effect curves		Difference between log C for drugs evoking 50% effect in Tyrode solution and depolarizing solution	pA_{10} for atropine (in Tyrode solution)	P (relative to pA_{10} for acetylcholine)
	in Tyrode solution	in depolarizing solution (M±m)	in depolarizing solution relative to maximum for acetylcholine in Tyrode solution	in Tyrode solution	in depolarizing solution			
Acetylcholine	1.0	1.0	0.87	0.16	0.25	2.30	8.63	
Aceclidin	0.98	0.87±0.026	0.75	0.32	0.39	1.15	8.66	> 0.5
Arecoline	0.99	0.80±0.026	0.70	0.37	0.38	1.75	8.73	> 0.5
Carbachol	1.05	1.0±0.025	0.87	0.23	0.25	1.45	8.93	0.1 > P > 0.05
Furmethide	0.97	0.94±0.017	0.82	0.2	0.36	1.15	8.75	> 0.5
Pilocarpine	0.96	0.27±0.034	0.23	0.38	0.36	·0.05	8.67	> 0.5

Depending on the magnitudes of their maximal effects in the latter case, the cholinomimetics studied can be arranged in the following series: acetylcholine–carbachol ≥ furmethide ≥ aceclidin ≥ arecoline > pilocarpine. The differences between acetylcholine and the last four members of the series were significant.

The observed difference between the maximal effects in Tyrode solution and in depolarizing solution can be attributed to one of the following causes: 1) the drugs act on two receptors, one of which does not function in depolarizing solution; 2) the smooth muscles of the stomach have a reserve of M-cholinergic receptors which does not function in depolarizing solution; and 3) the maximal effect is not directly proportional to the internal activity of the drug.

The first of these possibilities can be ruled out on the basis of a number of observations. Assuming that the receptors of the second type, not functioning in depolarizing solution, are ganglionic cholinergic receptors, it must be accepted that pilocarpine, of all the drugs tested, must have the greatest action because the maximal effect of pilocarpine was in fact reduced by the greatest degree in the depolarizing solution. On the other hand, the effects of acetylcholine, a drug whose ganglion-stimulating action can be quite strong, were not reduced in the presence of hexabenzoate, even in a concentration of 10^{-4} g/ml.

Finally, the values of pA_{10} obtained for atropine, as an antagonist of each of the agonist drugs tested, are in good agreement (Table 1), indicating the identity of the receptors stimulated by these drugs [8], and ruling out the possibility that the tested cholinomimetics act on two types of receptors.

There were likewise no grounds for postulating any reserve of receptors which could account for the maximal effects of the drugs in Tyrode solution. If a reserve of receptors functioning in Tyrode solution had been blocked when the strip of gastric smooth muscles was placed in depolarizing solution, the gradients of the curves obtained in depolarizing solution would have been less than the gradients of the curves obtained in Tyrode solution. However, the experiments showed that the slopes of the curves either were unchanged or were increased (Table 1). The shift of the curves to the left on the change from depolarizing to Tyrode solution, had a reserve of receptors existed under these conditions, must have correlated with the internal activity of the drugs (i.e., with the magnitude of the maximal effect) [4]. However, no correlation was found between these two indices ($r = 0.55$; $P > 0.05$), but a significant negative correlation was found ($r = -0.94$; $P < 0.016$) between the magnitude of the maximal effect in depolarizing solution and the gradient of the curves in Tyrode solution. Had a reserve of receptors been present, the correlation between these parameters must have been positive. Hence, the investigated cholinomimetics act on receptors of one type in the smooth muscles of the rat stomach, and no reserve of receptors is present. Since all drugs produced an identical maximal effect when all the receptors were occupied in Tyrode solution, but produced substantially different maximal effects in depolarizing solution, it must be accepted that the maximal effect is not directly proportional in magnitude to the internal activity of the drug and cannot serve as a measure of it.

The reason for the differences between the magnitudes of maximal contractions under the experimental conditions described is probably the multiplicity of pathways of pharmacomechanical coupling [9]. In isotonic potassium sulfate solution the smooth muscles were depolarized and an electrical coupling pathway was ruled out [5]. Under these circumstances only a contraction due to liberation of the calcium stored in the cell could take place [6]. Comparison of the maximal effects of the cholinomimetics (Table 1) leads to the conclusion that they differ in their ability to excite different coupling pathways. The ability to evoke a contraction through the liberation of calcium was strongest in the case of acetylcholine and carbachol and weakest in the case of pilocarpine. Meanwhile, the equality of the maximal effects of these drugs in Tyrode solution indicates that pilocarpine is quite capable of exciting the electrical coupling mechanism. Consequently, in a series of agonists acting on the same receptor, ability to excite different coupling pathways may differ. This discovery means that it is doubtful whether the ability of drugs to activate a receptor can be adequately defined by one single parameter (internal activity, effectiveness) by comparison between the values of observed effects [4, 10], without analysis of the actual coupling pathways concerned and of their relative importance in the mechanism of action of the agonists.

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