ANTAGONISTIC EFFECT OF CHOLINE-POTENTIATING DRUGS ON MUSCLE RELAXANTS

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Experiments on frogs and rats have shown that the anticurare and, in particular, the antiparamyon activity of choline-potentiating drugs is not entirely dependent on the anticholinesterase action of the compounds. It is postulated that the antagonism to muscle relaxants is partially due to their choline-sensitizing action.

The suggestion has been made [13, 19, 22] that the anticurare effect of choline-potentiating (anticholinesterase) drugs is not due entirely to inhibition of cholinesterase (CE), but also to a nonanticholinesterase action.

In the present investigation antagonism was studied between therapeutic choline-potentiating agents not only with D-tubocurarine, but also with its Soviet substitute paramyon [2].

EXPERIMENTAL METHOD

In experiments on male frogs all the compounds were injected subcutaneously. Choline-potentiating drugs were injected 10 min after atropine (1 mg/kg) and 1 min before D-tubocurarine (2 mg/kg) or paramyon (2.5 mg/kg), which, in control experiments, when injected into frogs rendered the animals unable to turn over when placed on their back for more than 20 min. The antagonistic effect on the muscle relaxants was regarded as positive if the paralysis lasted not more than 2 min. The choline-potentiating activity of the compounds was expressed as the negative logarithm of the concentration in which, acting for 100 min, the sensitivity of the isolated frog rectus abdominis muscle to acetylcholine (AC) was increased by 10 times (pP_{10}).

In experiments on male rats anesthetized with urethane (1 g/kg, intraperitoneally) and injected with atropine (10 mg/kg, intraperitoneally), paramyon in a dose of 1.1 mg/kg intravenously produced paralysis lasting for 6-20 min. The moment of onset of paralysis was determined from cessation of movement of a flag inserted into the diaphragm. The animals were kept alive during paralysis of their respiratory muscles by artificial respiration [6]. The effect of the choline-potentiating drugs was regarded as positive if, when injected at the time when the diaphragm stopped contracting, the paralysis was abolished in the course of the next 5 min. Minimal doses abolishing the paralysis in each of two experimental rats were determined ($ED_{2/2}$ [5]).

Anticholinesterase activity was determined by a colorimetric method [11] and expressed as negative logarithms of concentrations necessary to inhibit hydrolysis of AC by one-half ($pI_{0.5}$). Total CE of frog thigh muscles (substrate AC) and acetyl-CE of rat brain (substrate mecholine) were used. The CE activity of the rats' diaphragm was determined histochemically [14] 5 min after injection of the compounds in effective doses, and expressed by a conventional six-point scale.

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TABLE 1. Indices of Activity of Choline-potentiating Agents in Experiments on Frogs and Rats

Animals	Activity investigated and unit of measurement	Neostigmine	Oxacil	Armin	Galanthamine	Phosphacol	Eserine
Frogs	Anticurare activity (ED_{50} in mmoles/kg)	1.2	2.7	4.3	9.1	10.3	75.0
	Antiparamyon activity (ED_{50} in mmoles/kg)	1.2	–	2.5	2.7*	–	–
	Anticholinesterase activity on muscle CE ($pI_{0.5}$)	6.8	8.1	7.6	5.3	8.1	6.1
	Choline-potentiating activity on frog	5.2	6.8	6.2	4.6	4.9	2.6†
Rats	Antiparamyon activity ($ED_{2/2}$ in mmoles/kg)	0.12	1.6	0.19	13.0	0.36	0.75
	Anticholinesterase activity on acetyl-CE ($pI_{0.5}$)	6.0	8.6	7.7	5.6	8.3	6.8
	Inhibition of diaphragm CE (in points)	1.4	4.4	1.6	3.2	4.0	2.8

*Maximal effect 28%.
†Calculated by extrapolation.

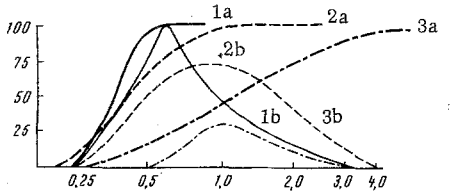

Fig. 1. Relationship between antagonism to muscle relaxants and dose of choline-potentiating drugs in experiments on frogs. a) Anticurare effect; b) antiparamyon effect; 1) effect of neostigmine; 2) of armin; 3) of galanthamine. Abscissa, doses of choline-potentiating compounds (in mg/kg); ordinate, frequency of prevention of paralysis (in percent).

EXPERIMENTAL RESULTS

The results of the experiments on frogs are given in Table 1. They show absence of correlation between anticurare and anticholinesterase activity ($r = 0.4$), whereas correlation between the anticurare and choline-potentiating activities is high ($r = 0.96$). It has previously been shown [8] that the potentiating effect of the frog rectus abdominis muscle is due not only to inhibition of CE, but also to a choline-sensitizing action. This suggests that the anticurare effect is due partially to a choline-sensitizing action.

The antiparamyon activity differed from the anticurare activity not only in that it was exhibited by only some compounds, but also that it was weakened by an increase in dose (Fig. 1). These differences are undoubtedly due to the character of the action of paramyon which, unlike curare, possesses not only an antipolarizing, but also a depolarizing effect [4]. The antiparamyon effect evidently cannot be attributed to inhibition of CE. Relatively weak CE inhibitors are active paramyon antagonists, whereas strong CE inhibitors (phosphacol and oxazil) not only do not abolish, but may actually prolong paramyon paralysis. These findings are in agreement with the view of Podlesnaya [3] that substances acting mainly through CE inhibition can potentiate the effect of paramyon by inhibiting its hydrolysis. It is highly significant that an antiparamyon effect is observed precisely in those compounds which have a marked choline-sensitizing action [7]. This confirms the hypothesis that antagonism to muscle relaxants may largely be determined by choline-sensitizing action.

The choline-sensitizing action is exhibited after administration of smaller doses than the anticholinesterase action [10], so that with an increase in dose the antagonism between choline-potentiating agents and paramyon is replaced by synergism.

In experiments on rats, the antiparamyon activity of the compounds did not coincide with their activity in experiments on frogs, presumably because of species-specific differences. These may be based on the more marked facilitatory action of choline-potentiating agents in warm-blooded animals [9, 13]. Correlation between antiparamyon and anticholinesterase activity was also absent in the experiments on rats ($r = 0.2$). No agreement likewise was observed in the degree of inhibition of the diaphragm CE, despite injection of equally effective doses of the compound.

The absence of correlation between anticholinesterase activity and antagonism to muscle relaxants indicates that this effect of choline-potentiating compounds is due partially to their nonanticholinesterase action. It has been shown previously that the cholinomimetric action does not play any significant role in the anticurare effect [16]. The facilitatory action, which probably plays an important role in the anticurare and, perhaps, in the antiparamyon action on warm-blooded animals [17], in the opinion of highly competent investigators [20], is not the only nonanticholinesterase mechanism abolishing the block to neuromuscular conduction. The effect of tetraethylammonium is produced entirely through this facilitatory action [15]. Experiments with D-tubocurarine-C^{14} have shown that curare paralysis can be abolished without displacement of the muscle relaxant from the cholinergic receptors of the muscles [21].

The results of this investigation suggest that the blocking of neuromuscular conductivity without liberation of receptors occupied by the muscle relaxant may be based on a choline-sensitizing action. Recovery of conductivity through the neuromuscular synapse on account of this choline-sensitizing action may take place through the recruiting of some of the reserve [18] receptors into synaptic transmission. For compounds possessing neither anticholinesterase nor facilitatory action, for example, for nikethamide or imidazole [1, 12], the sensitizing action may be the sole cause of antagonism to muscle relaxants. The anticurare effect of choline-potentiating agents in the experiments on cold-blooded animals was evidently determined both by their anticholinesterase and their choline-sensitizing action. In experiments on warm-blooded animals, their facilitatory action was added to the others.

The different types of action of choline-potentiating agents when used as antagonists of muscle relaxants differ in their importance. It can be assumed that the choline-sensitizing action is more important, and the anticholinesterase action less important, in the antiparamyon effect than in the anticurare effect.

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