N-Carbamoyl-2-(2,6-Dichlorophenyl) Acetamidine Hydrochloride (LON-954), a Tremorogen, on Rat Diaphragm

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LON-954, a synthetic benzylimidolylurea derivative, has been reported to produce reproducible and reversible tremor in a variety of animal species by a different mechanism than that of oxotremorine, the well known tremorogenic agent, in that it is devoid of central cholinergic involvements (1).

We earlier reported involvement of the skeletomotor apparatus in oxotremorine-induced tremor (2-4). Thus it was felt worthwhile to examine the influence of LON-954 on the skeletal myoneural system by employing innervated and denervated rat diaphragm preparations to determine the pre- and post-synaptic effects, if any.

Isolated phrenic nerve-diaphragm preparations were made from albino rats (150 to 200 g) of either sex according to the method of Bülbring (5) as described earlier by us (3).

For denervated diaphragm preparations, the left phrenic nerve was cut approximately 5 mm from the diaphragm through an incision between the 7th and 8th ribs (6). The rats were sacrificed between the 14th and 21st day after the operation, and their left hemidiaphragm was set up for direct stimulation by attaching two thin platinum electrodes in the diaphragm. Supramaximal square-wave pulses of 0.2 ms duration at a frequency of 0.2 Hz were used both for direct and indirect stimulation. At least 6 experiments were performed to determine the effect of a single dose of LON-954 and its interaction with drugs.

At lower concentrations (0.05 to 0.2 mM), LON-954 produced facilitation of indirect twitch responses of the diaphragm, the extent of which varied considerably in different experiments. Prior incubation with facilitatory concentrations (0.05 to 0.2 mM) of LON-954 potentiated the submaximal paralytic effect of d-tubocurarine chloride (0.8 \muM), thus indicating that the facilitation is not mediated through an anticholinesterase action of LON-954.

Incubation with increasing concentrations (0.35 to 1.4 mM) of LON-954 caused a graded blockade of indirect twitch responses (Fig. 1). The paralytic effect of LON-954 could neither be antagonized by prior incubation (1.5 \muM) with nor could be reversed by physostigmine sulfate (1.5 \muM), an anticholinesterase agent. In some experiments the paralytic effect of LON-954 was intensified in the presence of physostigmine.

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Fig. 1. Histogram showing dose-dependent blocking effect of LON-954 on indirect twitch responses of rat diaphragm. Values indicate the mean of six experiments±S.E.
This indicates that the neuromuscular paralytic effect of LON-954 on indirect twitch responses was not mediated through a competitive blocking action, but probably through the depolarizing action of this agent.

In an attempt to elucidate the possible mode of action, the influence of LON-954 was examined on the direct twitch responses of the denervated diaphragm, as well as on the responses of the denervated muscle to exogenous acetylcholine chloride and potassium chloride.

The criteria for complete denervation of the rat diaphragm was established by a) failure of d-tubocurarine (up to 8 μM) to produce any blocking action on the direct twitch responses of the diaphragm and b) ability of acetylcholine (2.5 to 10 μM) to produce dose-dependent contracture of the diaphragm resulting from the supersensitivity of acetylcholine receptors consequent to denervation (7). Similar to its effect on innervated diaphragm, LON-954 (0.35 to 1.4 mM) produced a concentration-dependent blocking effect on the directly elicited twitch responses of the denervated diaphragm. At a time when the paralytic effect of LON-954 on direct twitch responses was at a maximum (10 min after LON-954 administration), the responses to exogenous acetylcholine (2.5 to 10 μM) were blocked, whereas the responses to exogenous potassium chloride (0.025 to 0.1 M) were potentiated (Fig. 2). This indicates that LON-954 does not impair excitation-contraction coupling since the suppression of twitch tension caused by dantrolene, an established excitation-contraction uncoupler, has been reported to be accompanied by a reduced potassium contracture (8). The paralytic effect of LON-954 on direct twitch responses was preceded by a contracture of the diaphragm upon incubation with 1.4 mM LON-954 (Fig. 2).

Prior incubation with d-tubocurarine chloride (0.8 μM) completely prevented the contracture as well as the blockade of direct twitch responses of the denervated diaphragm induced by 1.4 mM LON-954, thus showing that LON-954 is an agonist as well as an antagonist.

The mechanism of action of the neuromuscular effects of LON-954 appears to be a complex one having more than a single component. That LON-954 possesses a depolarizing property was indicated by its ability to produce contracture of the diaphragm at high concentrations which was prevented in the presence of d-tubocurarine. The paralytic effect of LON-954 on direct twitch responses of denervated diaphragm may be a result of prolonged depolarization of the denervated muscle fiber since in a different set of experiments it was found that succinylcholine, a depolarizing agent, also inhibited direct twitch responses of denervated diaphragm.

Since the same concentration of LON-954 could produce blockade of indirect and direct twitch responses in the present study, LON-954 possessing either a local anaesthetic action or a presynaptic action is ruled out. Furthermore, this observation suggests that LON-954 at higher concentrations probably inhibits the generation of action potentials at the membranes of the muscle and nerve fibers. It is plausible to conclude that LON-954 possesses the following multotropic actions at the skeletomotor site: 1) Inhibition

Fig. 2. Effect of LON-954 on direct twitch responses and on responses to acetylcholine (ACh) and potassium chloride (KCl) of the denervated rat diaphragm preparation (W=wash).
of action potential generation at the membranes of the muscle and nerve fibers. 2) Actions on the acetylcholine receptors as an agonist as well as an antagonist. However, at present no explanation could be provided for the potentiation of potassium chloride-induced contracture of the denervated diaphragm in the presence of LON-954.

The present study shows that the skeletal myoneural site is affected by LON-954, like oxotremorine, which may contribute in its tremorogenic action. The question of whether or not LON-954 also affects the other peripheral and spinal sites of oxotremorine action, for example, the intrafusal myoneural apparatus (4, 9) and the recurrent inhibition in the spinal cord (10), are currently being examined in order to identify the common sites that may be involved in tremorogenesis.

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