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The use of neuromuscular blocking agents to investigate receptor structure requirements for histamine release

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The polymeric compound 48/80 has been used most frequently to demonstrate histamine release in experimental animals, but recent work has shown that the mechanism of the release from isolated rat peritoneal mast cells by tubocurarine was similar to that of 48/80 (Frisk-Holmberg and Uvnäs, 1969). It was of interest, therefore, to investigate several other neuromuscular blocking agents for their ability to release histamine, in an attempt to determine the molecular features necessary for histamine liberation.

Histamine release was measured in rat mast cells using a fluorimetric method (Frisk-Holmberg & Uvnäs, 1969), in the guinea-pig by a broncho-constriction test (Konzett & Rössler, 1940) and in isolated perfused cat paws, after the following clinically used neuromuscular blocking agents: tubocurarine chloride, dimethyltubocurarine chloride, C-toxiferine chloride, alcuronium chloride, pancuronium bromide (Buckett, Marjoribanks, Marwick & Morton, 1968) and dacuronium bromide (Buckett & Saxena, 1969). Neuromuscular blocking potency was also determined in anaesthetized cats and conscious mice after intravenous administration, but no correlation between muscle relaxation and histamine liberation was found.

Tubocurarine was the most potent releaser of histamine in all species, whereas pancuronium was almost inactive. For example, in rat peritoneal mast cell suspensions, tubocurarine (10^{-3}M) released 57·8% of total histamine content in contrast to the 2% liberated by pancuronium (Table 1).

The rank order of potency of the neuromuscular blocking agents in releasing histamine was similar for the three test situations, allowing the following conclusions to be drawn.

Methylation of the two phenolic hydroxyl groups of tubocurarine to dimethyltubocurarine reduced the ability to release histamine. Both C-toxiferine and alcuronium contain two primary hydroxyl groups, but the moderate histamine releasing

TABLE 1. *Release of histamine from rat peritoneal mast cells by neuromuscular blocking agents*

		Molar concentration			
Drug	*n*	10^{-4}	5×10^{-4}	10^{-3}	5×10^{-3}
Tubocurarine	(5)	16·33±1·69	40·50±3·58	57·80±5·01	64·50±1·97
Dimethyltubocurarine	(6)	7·66±1·56	20·00±0·54	33·17±1·79	37·67±1·16
Toxiferine-C	(4)	5·25±1·08	13·75±0·28	23·25±1·48	29·50±0·50
Alcuronium	(4)	2·00±0·20	6·75±0·08	15·25±0·88	20·25±0·88
Dacuronium	(4)	0	2·75±1·59	5·00±2·25	7·25±2·59
Pancuronium	(4)	0	0	2·00±0·25	4·00±0·25

The figures represent percentage release of total histamine content (±S.E. of mean) corrected for spontaneous histamine release (less than 1%); *n*= number of determinations.

ability of the former is reduced by allyl quaternization, the double bonds of which might form hydrogen bonds with the hydroxyl groups and thus reduce access of the latter to the receptor site. The weak histamine releasing power of dacuronium is practically abolished on acetylation of its 17-hydroxy group to give pancuronium. From this evidence it appears that one or more free hydroxyl groups, either primary, secondary or phenolic in nature, enhance histamine release. In addition, the interonium distance in these compounds may also play a critical part.

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The Schultz-Dale reaction in bovine pulmonary smooth muscles

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Calves 1–4 months old were sensitized by whole ovalbumin or horse serum (alone or with Freund's complete adjuvant), or ovalbumin-alum precipitate. At least 3 weeks after sensitization, animals were killed with pentobarbitone and the pulmonary artery, vein and bronchus taken from a lung with minimum delay. The blood vessels were cut spirally and the bronchus transversely into rings before mounting in a bath of 20 ml oxygenated Krebs solution at 35° C. Contractions were recorded isotonically on a pen recorder.

The pulmonary artery contracted to 5-hydroxytryptamine (5-HT; 10–20 ng/ml) and histamine (40–2,000 ng/ml). The pulmonary vein was at least 20 times more sensitive to these drugs; sometimes contracting to as little as 0·1 ng 5-HT/ml (Fig. 1). The bronchus, however, was 10–100 times less sensitive than the artery (confirming the observation of Aitken & Sanford, 1970).

Among sixteen "sensitized" calves, fourteen showed positive Schultz-Dale reactions in pulmonary veins to 50–500 μg ovalbumin or 0·1–1·0 ml horse plasma (Fig. 1). Plasma was used since serum contained enough 5-HT *per se* to contract the vein. Repeated antigen caused diminishing responses (desensitization). Of the fourteen animals giving positive pulmonary vein reactions, seven were also challenged in the artery and bronchus. Four such arteries and two bronchi gave feeble Schultz-Dale reactions. On two occasions strips of ileum were set up (Bywater, 1969) and these failed to contract to antigen.

The results support the contention that the lung is an anaphylactic shock organ of domesticated ruminants, but focus attention on the pulmonary vein rather than the artery or bronchus cited previously (Aitken & Sanford, 1969, 1970; Alexander, Eyre, Head & Sanford, 1970). We are presently making detailed studies of the Schultz-Dale phenomenon in cattle and have completed a similar study in sheep.