Effects of neuromuscular blockers on carnosine levels in rat skeletal muscle[1]

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Summary. A 30-min treatment with neuromuscular blocking doses of either physostigmine or d-tubocurarine was associated with a 44% or 36% (respectively) reduction in rat skeletal muscle carnosine levels in vivo.
Key words. Rat muscle; neuromuscular blocking; carnosine; physostigmine, d-tubocurarine.

The dipeptides carnosine (β-alanyl-L-histidine) and anserine (β-alanyl-1-methylhistidine) are two of the most abundant nitrogen containing compounds associated with mammalian skeletal muscle[4]. During development of electrochemical coupling in skeletal muscle, carnosine and anserine appearance has been reported to coincide with increased concentration of acetylcholine (ACh) and acetylcholinesterase at the neuromuscular junction[5]. Higher concentrations of carnosine are found associated with nerve-rich portions of skeletal muscle[6] and denervation of rat skeletal muscle has been reported to be associated with a reduction in carnosine levels[6,7]. Carnosine has been reported to increase the duration and amplitude of muscle contraction as well as restore muscular function after curare blockade or fatigue[6]. Stimulation of ACh synthesis in insect muscle by imidazole and carnosine has been reported[8]. These observations indicate that carnosine may be associated with the acquisition and normal functioning of electrochemical coupling in skeletal muscle.

In this investigation the in vivo influence of various cholinergic drugs on carnosine and anserine levels of rat skeletal muscle was determined. Carnosine levels were rapidly decreased with neuromuscular blocking doses of d-tubocurarine and physostigmine.

Materials and methods. Atropine sulfate, carbamyl choline chloride (carbachol), carbamyl β-methylcholine chloride (bethanechol), eserine sulfate (physostigmine), and d-tubocurarine HCl were purchased from Sigma (St. Louis, MO). [^{3}H]-Carnosine was enzymatically prepared with [^{3}H]-β-alanine (Amersham) and L-histidine as described by Ng and Marshall[9]. Dowex AG50X4 cation exchange resin was obtained from BioRad (Richmond, CA). 2,6-Lutidine (2,6-dimethylpyridine) was purchased from ICN Life Sciences Group (Cleveland, OH). All other reagents used were of the highest grade available. Male, 5-week-old rats were purchased from Sasco Live Animals (Omaha, NE).

Atropine, physostigmine, and d-tubocurarine were injected i.p. in a volume of 0.5 ml and treatment was for 30 min, unless stated otherwise. Bethanechol, and carbachol, were administered s.c. in a volume of 0.5 ml and treatment lasted for 90 min. Control groups were injected with 0.5 ml of isotonic saline either i.p. or s.c. The drug doses and periods of treatment used were as described by Barnes and Eltherington[10] and elicited the pharmacological actions desired. With a dose of 1.0 mg/kg physostigmine there were muscle tremors (fasciculations) and a lack of movement, but no respiratory failure. No effects were observed with a dose of 0.1 mg/kg. With a dose of 0.3 mg/kg d-tubocurarine neuromuscular blockade was evident by their lack of movement; however, there was no respiratory failure. No effects were observed at a dose of 0.15 mg/kg of d-tubocurarine.

Male Sprague-Dawley rats (200 g) were sacrificed by cervical dislocation after the appropriate treatment interval. The entire hind leg muscle was rapidly removed, and the extraction of carnosine and anserine was performed as reported by Seely and Marshall[11]. Carnosine levels were determined spectrophotometrically as described by Van Balgooy et al.[12] and anserine levels were quantitated as described by Parker[13]. Carnosine was identified in rat skeletal muscle by the following criteria: 1) coelution with authentic [^{3}H]carnosine during Dowex AG50X4 cation-exchange chromatography; 2) comigration with authenic carnosine on silica gel 60 TLC sheets; 3) the material isolated gave positive diazo and ninhydrin reactions; 4) disappearance of original material and liberation of β-alanine and L-histidine following 24-h hydrolysis at 100 °C. Anserine was identified by a similar set of criteria.

Results and discussion. Rats receiving neuromuscular blocking doses of 1.0 mg/kg physostigmine or 0.3 mg/kg d-tubocurarine displayed reduced skeletal muscle carnosine levels (44% and 36% of controls, respectively) without any change in anserine levels (see table). Both of these drug treatments produced pronounced neuromuscular blockade within 5 min after i.p. injection and lack of movement persisted throughout the 30-min treatment period. However, rats receiving 0.1 mg/kg physostigmine or 0.15 mg/kg d-tubocurarine did not display decreased carnosine levels. At these lower doses, physostigmine and d-tubocurarine did not promote neuromuscular blockade which was observed at higher doses. Carbachol, 0.5 mg/kg, bethanechol, 25 mg/kg, and atropine, 50 mg/kg treatments as described in 'methods' were without effect on skeletal muscle carnosine and anserine concentrations (data not shown). Pretreatment of rats with a neuromuscular blocking dose of d-tubocurarine (0.3 mg/kg) for 10 min followed by a subsequent injection of 1.0 mg/kg physostigmine reversed initial paralysis (observed approximately 5 min after administration) elicited by d-tubocurarine and no reduction of carnosine was observed. However, a second injection of 0.1 mg/kg physostigmine which was not capable of reversing initial neuromuscular blockade elicited by d-tubocurarine was accompanied by a decrease of carnosine of the same magnitude as that elicited by the blockade producing dose of d-tubocurarine alone. These results indicate that neuromuscular blockade persisting for a relatively short period is accompanied by an alteration of carnosine concentration. In addition, when blockade was reversed 10 min after initial blockade, there was no alteration in carnosine.

The pharmacological actions of d-tubocurarine[14-16] and physostigmine[17] have been extensively reviewed. Physostigmine has been reported to have a 'decurarizing' action that will reverse the antagonism produced by competitive neuromuscular

Influence of physostigmine and d-tubocurarine on rat skeletal muscle carnosine levels

Drug treatment	n**	Dose (mg/kg)	Time (min)	Carnosine (μmoles/g wet wt)	Anserine (μmoles/g wet wt)
None	18	–	30	1.45 ± 0.38	4.46 ± 1.36
Physostigmine	18	0.10	30	1.53 ± 0.61	4.53 ± 0.83
		1.0	30	0.81 ± 0.15*	4.45 ± 0.99
d-Tubocurarine	18	0.15	30	1.52 ± 0.25	4.73 ± 1.29
		0.30	30	0.92 ± 0.43*	4.43 ± 0.72
d-Tubocurarine		0.30	30		
followed by	6	0.1	20	0.83 ± 0.23*	4.38 ± 0.71
physostigmine***	6	1.0	20	1.63 ± 0.61	4.59 ± 0.62

*Denotes means that are statistically different ($p < 0.01$) from the control mean. **Denotes the number of independent experiments. ***The first drug was injected, followed 10 min later by the second injection.

blockers[18]. It is not clear how physostigmine or d-tubocurarine induced neuromuscular blockade could influence muscle carnosine levels after such a short period of drug treatment. The half life of carnosine in rat skeletal muscle has been reported to be greater than 29 days[19]. Our data does not indicate any change in anserine levels with these treatments. The exact significance of our observations is unclear, and additional experiments are needed to determine if they may support the premise that carnosine may be involved with the maintenance of normal electrochemical coupling in mammalian skeletal muscle.

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H_2 Receptor antagonists and human granulopoiesis[1]

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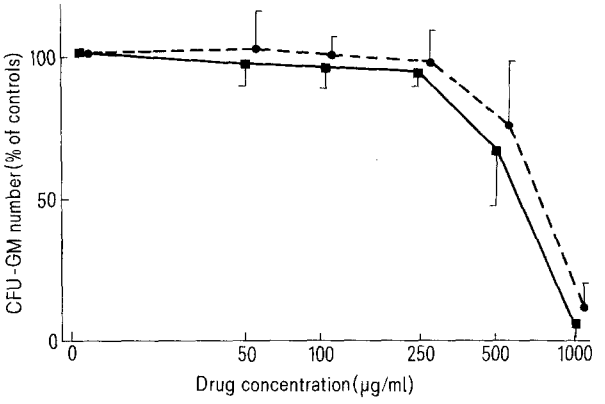
Summary. The effect of 2 H_2 receptor antagonists (ranitidine and cimetidine) on the in vitro growth of human granulomonopoietic precursors (CFU-GM) was studied. Ranitidine, although having an anti H_2 receptor activity much greater than that of cimetidine, displays the same toxicity for CFU-GM.
Key words. Granulopoiesis, human; H_2 receptor antagonists; ranitidine; cimetidine; granulomonopoietic precursors, inhibition of.

A certain degree of hematopoietic toxicity has been observed with the H_2 receptor antagonists used in the treatment of peptic ulcer. Metiamide, indeed, has been responsible for several cases of marrow aplasia and has, therefore, been withdrawn. Its substitute, cimetidine, has a cyanoguanidine group instead of the thiourea group thought to be the cause of this toxicity[2], but has nevertheless been incriminated in numerous cases of neutropenia and some instances of aplasia[3-5]. It has also been regarded as potentiating the myelosuppression induced by cytostatic treatment[6]. Interaction with the H_2 receptors on hemopoietic stem cells has been put forward as the explanation of this marrow toxicity[5]. Both metiamide and cimetidine, in fact, block the entry of resting pluripotent murine stem cells (CFU-S) into the cell cycle. In the human, metiamide blocks the 4-methylhistamine-induced entry of resting precursors of CFU-GM into the cycle[7]. H_2 receptor blocking is also suggested as an explanation for their in vitro inhibition of human granulo-monocyte precursors[8].

To determine whether this inhibition is really dependent on interaction with H_2 receptors we studied the effect of ranitidine and cimetidine on the growth of CFU-GM in vitro. Since the anti H_2 receptor activity of ranitidine is 4–6 times greater than that of cimetidine[9-11], it was expected to prove a proportionally more powerful inhibitor of growth if the inhibition depended solely on receptor block.

Material and methods. Normal CFU-GM cultures were prepared as described elsewhere[12]. Briefly, marrow blood samples from patients subjected to check surgery for non-neoplastic diseases were collected in preservative-free heparin, diluted with isotonic saline, and stratified on lymphoprep (density 1,077, Nyegaard, Oslo, Norway). The light density fraction was collected and washed 3 times. One $\times 10^5$ cells, resuspended in McCoy's 5A medium (supplemented with essential and non-essential amino acids, sodium pyruvate and glutamine) +15% fetal calf serum were seeded on 0.3% agar gel in 35 mm Petri dishes. Increasing concentrations of ranitidine (Zantac, Glaxo, Verona, Italy) or cimetidine (Tagamet, Smith, French & Kline, Milan, Italy) were added to these cultures. Colony growth was stimulated with 10% GCT condi-



Effect of increasing concentrations of cimetidine ■——■ and ranitidine ●- - -● on in vitro growth of normal CFU-GM (9 samples). Values are expressed as percentages of control colony number. Results are means ± SD of these percentages. Colony number in control dishes/10^5 light density cells: 82–198.