Vagotomy and atropine antagonism of (±)-chloroacetyl carnitine chloride mediated blood pressure reduction in the rat

Carnitine and carnitine derivatives have been reported to possess cholinergic activity (Dallemagne, Philippot & others, 1955; Yoshimi, Takaori & Shimamoto, 1965). Experiments now reported suggest that (±)-chloroacetylcarnitine chloride [$Me_3N^+ \cdot CH_2 \cdot CH(CH_2 \cdot COOH)O\text{-}COCH_2Cl \cdot Cl$; CAC] produces cholinergic-like effects on rat blood pressure *in vivo*. Furthermore, blood pressure reduction is antagonized by vagotomy and by atropine pretreatment.

CAC (28 mg/kg, i.v.) significantly reduces blood pressure of 200–250 g female albino Sprague Dawley rats (Table 1). After vagotomy, about five times the dose is required to reduce blood pressure by 13%; after atropine pretreatment, administration of approximately half the dose which produces 50% lethality elicits a non-significant 4% reduction in blood pressure.

The data show that CAC possesses acetylcholine-like activity as reported for other acetyl carnitine derivatives (Dallemagne & others, 1955; Fritz, 1963; Yoshimi & others, 1965). Blood pressure reduction does not appear to be mediated by ganglionic blockade since CAC did not inhibit the response of the cat nictitating membrane to pre- and post-ganglionic sympathetic electrical stimulation. Antagonism of CAC-produced blood pressure reduction in atropine treated and vagotomized rats suggests central mediation of the effect through cholinergic mechanisms. Since passage of these quaternary ammonium compounds into the brain may be difficult, a post-ganglionic site of action of CAC may be an alternative hypothesis.

Table 1. *Antagonism of (±)-chloroacetylcarnitine chloride fall in blood pressure by vagotomy and atropine.* Each value represents the mean of at least two determinations. Animals were anaesthetized with sodium pentobarbitone (35 mg/kg, i.p.). Carotid artery blood pressure was monitored using the E & M Physiograph. The test compound was administered into the jugular vein. Responses of all preparations were standardized with methacholine, acetylcholine and adrenaline.

Intact		Vagotomized		Atropine pretreated	
Dose (mg/kg)	Decrease %	Dose (mg/kg)	Decrease %	Dose (mg/kg)	Decrease %
7·1	0	28·4	4	14·0	0
14·2	13	35·7	11	29·0	15
28·4	53	71·4	13	36·0	0
42·6	33			72·0	0
				171·0	4

(±)-Carnitine and (±)-acetylcarnitine are converted to β-methylcholine by carnitine decarboxylase in liver, muscle and kidney rat mitochondrial preparations (Khairallah & Wolf, 1967). Thus biotransformation of CAC may be important in mediation of the reported cholinergic effect, since perfusion of rat hand limb vasculature with CAC results in reduced flow (Louis-Ferdinand, Cutroneo & others, 1970).

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Uptake of [7, 8-^{3}H]dihydromorphine by rat cerebral cortical slices and eye tissue

Morphine and its congeners have recently been reported by Scrafani & Hug, (1968) to be accumulated in rat cerebral cortical slices by an active transport process. We now report an inability to saturate the uptake system at concentrations ranging from 2·5 to 20·0 μg/ml likely to be encountered *in vivo* and the inability of glucose-substrate or metabolic inhibitors to depress the uptake of [7,8-^{3}H]dihydromorphine.

Male Holtzman rats, 140–200 g, were decapitated, the brains placed in a cold chamber and tissues sliced by an apparatus (O'Neill, Simon & Cummins, 1963) adjusted to give two outer slices, one dorsal and one lateral (weight, 15–25 mg, thickness, 0·2 mm) from each hemisphere. The slices were transferred to incubating beakers (20 ml) containing 2 ml oxygenated calcium-free Ringer (Elliot, Kokka & Way, 1963), previously kept in ice, and dihydromorphine, and incubated in Dubnoff metabolic shaker at 37° under oxygen with a shaking rate of 140 strokes/min. Control samples prepared as above were kept at 0° without shaking. At the end of the incubation, beakers were quickly placed on ice, tissue slices rinsed several times with saline, transferred to tared aluminium foil and dried to a constant weight at 105° and their radioactivity assayed.

The accumulation of dihydromorphine (2·2 μCi/mg) by slices in oxygen at 37° reached a steady-state distribution between tissue and medium in about 30 min (Fig. 1a). The uptake of dihydromorphine by slices was linear and unsaturable at concentrations of 2·5 to 20 μg/ml (Fig. 1b). The tissue/medium ratio (T/M) showed slight changes as dihydromorphine concentration was increased in the incubating solution. These findings were not altered with the omission of glucose from the media. At low concentrations of dihydromorphine (1·25 μg/ml) the T/M ratio was greater than at other concentrations studied. Neither the addition of calcium ($1{\cdot}3 \times 10^{-3}$M) to incubating solution nor 3-day fasting of animals changed the effect of glucose on the uptake of dihydromorphine. Glucose had no effect on accumulation of dihydromorphine in tissue slices when the concentration of dihydromorphine was 1·25 μg/ml. Increasing glucose concentrations from 0 to 12 mM at 37° produced a corresponding decrease in dihydromorphine uptake, which could not be accounted for on the basis of slight pH changes alone during incubation for 30 min.

Dinitrophenol, nitrogen atmosphere and high potassium content in the medium (an additional 1×10^{-1}M) significantly inhibited the accumulation of dihydromorphine in the slices (Table 1). Sodium cyanide, sodium malonate, iodoacetate, fluoroacetate had no effect on accumulation of dihydromorphine in slices with or without glucose in incubating medium.

Although nalorphine at a concentration of 2·5 μg/ml and with a 15 min preincubation period before the addition of dihydromorphine to the medium, did not alter the accumulation of dihydromorphine in the tissue, pretreatment of rats with a subcutaneous injection of nalorphine, 20 mg/kg, 30 min before decapitation, produced a significant decrease in tissue accumulation of dihydromorphine when the incubating solution contained no glucose but there was no effect when glucose was in the medium.