Antagonistic activity of galanthamine on constant infusion of pancuronium in rats

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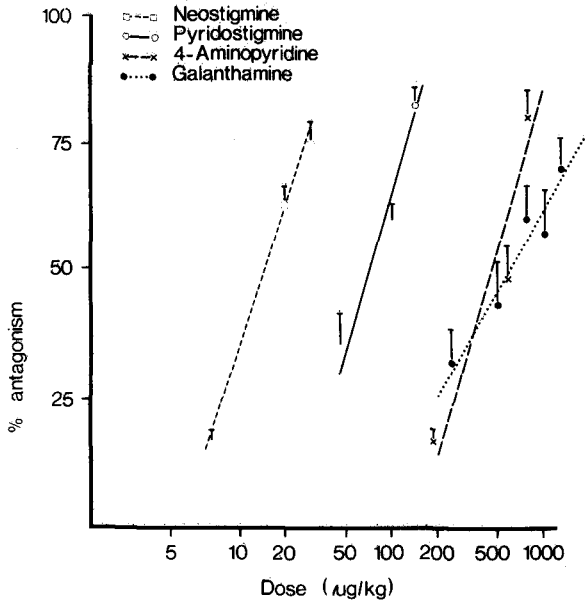
Summary. The effect of galanthamine, an anticholinesterase having a tertiary ammonium group, on a constant infusion of pancuronium was studied in the rat tibialis anterior-sciatic nerve preparation. The results were compared with those previously obtained with neostigmine, pyridostigmine, and 4-aminopyridine. The antagonizing activity of galanthamine is similar to that of 4-aminopyridine and less than that of the other 2 anticholinesterases. The duration of galanthamine antagonism is similar to that of neostigmine and pyridostigmine but less than that of 4-aminopyridine.

Galanthamine hydrobromide (Nivalin, Pharmachim) is an anticholinesterase containing a tertiary ammonium group. It not only antagonizes a non-depolarizing blockade[2-4] but may have advantages over physostigmine in reversing the cerebral side effects of antimuscarinic substances because of its longer duration of activity[5,6]. In the present study, this substance was administered in vivo to establish its effect on a constant infusion of pancuronium. The results were then compared to an earlier investigation[7] made using the same preparation in this laboratory.

43 male Wistar rats, 280–410 g, were anesthetized with urethane 500 mg kg^{-1} and pentobarbitone sodium (Nembutal, Abbott) 40 mg kg^{-1} i.p. Indwelling plastic cannulae were inserted into both jugular veins for the administration of drugs. Arterial blood pressure from the carotid artery, and twitch tension were recorded on a polygraph. A tracheostomy was performed to facilitate controlled ventilation with room air by a Braun air pump. The tendon of the left tibialis muscle was freed, sectioned and connected to a Grass F 0.3 force displacement transducer. The sciatic nerve was stimulated by supramaximal twitch stimulus of 0.2-msec duration through a bipolar electrode. Temperatures of the prepared muscle and rectum were monitored and maintained between 37 and 38 °C with heating lamps. Anesthesia was maintained by injecting pentobarbitone 3 mg i.v. or 6 mg i.p. Pancuronium bromide (Pavulon, Organon) was continuously infused to depress twitch tension to 85–95%. When the depression of twitch tension was constant for 8–10 min, galanthamine in doses varying from 0.25 to 2.2 mg kg^{-1} was administered i.v. 1 dose of galanthamine was given to each animal. The percent of antagonism, ED_{50} (dose of galanthamine which produced a 50% antagonism), onset (time from galanthamine injection to peak effect), and duration (time of galanthamine administration to 50% return) of the pancuronium-depressed twitch height were measured. The logarithmic dose response curve was compared with those made with neostigmine, pyridostigmine and 4-aminopyridine in an earlier investigation performed in the same laboratory[6].

Maximum antagonism was achieved by galanthamine, 1.3 mg kg^{-1} (table). Increasing the dose to 1.7 or 2.2 mg kg^{-1} produced no additional antagonism. There was no correlation between onset time and the dose of galanthamine. The duration of the galanthamine effect increased with increasing doses up to 1.0 mg kg^{-1}, but the 1.3 mg kg^{-1} dose resulted in a duration slightly more than that achieved by 0.50 mg kg^{-1}. The figure shows the effect of galanthamine (250–1300 µg kg^{-1}) on percentage antagonism. The linear relationship between the actual percentage and the $\log_{10}$ (dose) $y = a + bx$ where y = percentage response, $x = \log_{10}$ (dose), b = slope of line, and a = y intercept, was calculated. The correlation coefficient r, being 0.598,



The $\log_{10}$ dose response curve of galanthamine in comparison with neostigmine, pyridostigmine, and 4-aminopyridine. The data for the last 3 compounds are by courtesy of Prof. R. D. Miller and The Editor, Journal of Pharmacy and Pharmacology.

The effect of galanthamine on continuous infusion of pancuronium

	Galanthamine dose (µg kg^{-1}) 250	500	750	1000	1300	1700	2200
Pancuronium	93.7	90.0	93.0	90.6	91.5	89.4	90.8
block (%)	(0.4)	(0.9)	(1.4)	(0.8)	(0.6)	(0.0)	(2.0)
Antagonism (%)	33.0	44.0	61.2	57.8	71.8	76.5	78.0
	(6.6)	(9.0)	(6.6)	(8.6)	(5.2)	(6.9)	(8.0)
Onset to peak effect (min)	2.7	2.5	7.2	4.7	2.1	2.4	3.1
	(0.4)	(0.5)	(2.0)	(1.6)	(0.5)	(0.4)	(0.6)
Duration 50% (min)	11.6	20.8	32.1	38.0	22.5	28.8	37.9
	(0.9)	(4.3)	(3.8)	(6.3)	(4.2)	(3.5)	(4.4)

Values in brackets indicate SEM.

a = 63.014, b = 52.909, and ED_{50}, 0.568. On the same figure are plotted the results obtained previously in the same laboratory. The ED_{50} which produced a 50% antagonism of the pancuronium-depressed twitch tension were 18, 49, 440, and 600 μg kg^{-1} for neostigmine, pyridostigmine, 4-aminopyridine, and galanthamine respectively. Galanthamine provoked a 10–25% increase of systolic blood pressure in 34 (80%) of the animals. The effect lasted from 3 to 19 min. Onset time to peak effect with galanthamine was similar to that seen by Miller et al[7] with neostigmine, both of them exerting a more rapid onset than that of pyridostigmine and 4-aminopyridine. Duration of galanthamine antagonism was similar to that of neostigmine and pyridostigmine, but shorter than that of 4-aminopyridine.

On the basis of the study, and comparing the results with those of Miller and his colleagues[7], galanthamine is the least potent of the 3 cholinesterase inhibitors. Its potency is rather similar to that of 4-aminopyridine (fig.). However, the validity of this comparison might well be disputed on 2 points. Firstly, the log dose response curve for galanthamine was constructed around 5 points whereas those of the other compounds were on 3 points. Secondly, in our hands, unlike Miller et al., it was impossible to maintain the steady state of the deep pancuronium block before galanthamine administration for more than 8–10 min, whereas Miller et al. waited 15 min. The rise in blood pressure seen here could well have been prevented had atropine been given[8]. The anticholinergic agent was not injected because of reports stating that the muscarinic side effects of galanthamine are minimal[9].

In conclusion, a constant infusion of a non-depolarizing neuromuscular drug in the rat tibialis anterior-sciatic nerve preparation is a useful agent in comparing and assessing antagonists to muscle relaxants. Galanthamine was about 30 times weaker than neostigmine and this is in good agreement with the 1:18 ratio observed on the phrenic nerve preparation[4] and the ratio of 1:20 seen in human studies[3].

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Toxicity of Temik (aldicarb) for a fresh water teleost, *Barbus conchonius* Hamilton

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Summary. The toxic effects of Temik (aldicarb) on a fresh water Himalyan lake teleost, *Barbus conchonius* were investigated in hard and soft water. The 48-, 72- and 96-h TL_m-values in mg/l were 8.99, 2.39 and 2.42 respectively in the hard-water test and 3.30, 0.62 and 0.46 in the soft-water test. The toxicity of Temik to *B. conchonius* increases many fold in soft water.

The use of carbamates for the control of agricultural pests is a common practice, although it is well known that they are a source of aquatic pollution, hazardous for the life of piscine and non-piscine fauna. The literature hitherto available deals with the detrimental effects of carbamates such as carbaryl and mexacarbate[2,3]. Temik, a newer systemic pesticide belonging to the carbamate group, is in use to control agricultural pests[4]. It has been found to be toxic to beneficial insects[5] and birds[6], too. However, there is no information on the toxicity of Temik to fishes. On account of its high solubility in water (6000 ppm), Temik is liable to be washed into bodies of water. The present report deals with the acute toxic limits of Temik for a Himalyan lake teleost, *Barbus conchonius*, in hard and soft water; to the best of our knowledge it is the first on this subject.

Healthy specimens of *B. conchonius* (4.8 ± 0.45 cm in size), collected from the high altitude Himalyan lake Nainital, were acclimatized to laboratory conditions for at least 1 week in a quality of water similar to that in which the toxicity bioassays were carried out. During the acclimatisation, fishes were fed rice bran. The hardness in terms of $CaCo_3$ and pH of the water was 318.57 ± 1.6 and 7.41 in the case of hard water and 60.69 ± 0.15 and 7.16 in the case of soft water. The temperature variation during the period of experiments was between 14 and 22 °C. The bioassay experiments were performed in stationary water by exposing the fishes to various concentrations of Temik; between 0.25 ppm and 1.50 ppm for the soft-water test and 1.00 ppm and 6.00 ppm for the hard-water test. There was no food supply during the course of the experiment.

The mortality data collected at 28, 72 and 96 h were analyzed for the calculation of TL_m-values according to the procedure of Finney[7]. The comparative TL_m-values of Temik for *Barbus conchonius* in hard and soft water are

Comparative TL_m-values of Temik for *B. conchonius* in hard and soft water

Time (h)	Hard water No. of test animals	TL_m-values ± SE (mg/l)	95% confidence limits (mg/l)	Soft water No. of test animals	TL_m-values ± SE (mg/l)	95% confidence limits (mg/l)
96	180	2.42 ± 0.010	2.28–2.52	210	0.459 ± 0.0010	0.445–0.521
72	180	2.39 ± 0.011	2.18–3.951	210	0.623 ± 0.0012	0.492–2.692
48	180	8.99 ± 0.014	4.265–18.586	210	3.296 ± 0.0017	1.116–9.727