The effect of cholinergic antagonists on a central response to nicotine[1]

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Summary. Mecamylamine and hexamethonium antagonize the prostration response of rats to centrally-administered nicotine; decamethonium and d-tubocurarine are less effective. Physostigmine does not elicit the response. Nicotine's central cholinergic effect thus may contribute to but does not fully account for its action.

The prostration response of rats to intraventricularly injected nicotine has been proposed as evidence of a noncholinergic receptor for this alkaloid[2]. Crucial to this proposal was the observation that neither typical neuromuscular blockers (d-tubocurarine and decamethonium) nor muscarinic antagonists blocked this response; of the compounds assayed, only derivatives of nornicotine and piperidine proved to be effective antagonists. We wish to report that the ganglionic blockers mecamylamine and hexamethonium, not evaluated in the earlier study, are also highly effective antagonists; in addition, the neuromuscular blockers cited show slight to moderate activity at less than equimolar doses.

Materials and methods. Nicotine (free base, obtained from Aldrich Chemical Co.) was distilled under reduced pressure; all other drugs were obtained from Sigma Chemical Co. and used without further purification. Cannulations penetrating the lateral ventricle were carried out on male Sprague-Dawley rats (150–200 g weight) as described[2b] (vertical coordinate = 5.0). The compounds to be tested were dissolved in physiological saline in concentrations appropriate for a 10-µl injection; antagonists were administered 1 min before nicotine challenge (100 nmoles). Response was graded from 0 (no response) to 4 (completely prostrate and immobile) in half-point increments, 1 point for each limb immobilized and ½ point for tremor (tremors were not observed at full prostration, but younger rats occasionally exhibited 1–2 rotating convulsions of the vasopressin type[3]). An alternate-day injection regimen delays tolerance development (e.g., day 1, nicotine only; day 3, antagonist + nicotine; day 5, nicotine; etc.) rats not maintaining a nicotine response ≥ 3 were removed from the study. Percent antagonism was defined as 100%–100% ([response to antagonist + nicotine]/[average of the response to nicotine alone before and after test for antagonism]). Each animal thus served as his own control; at each dose, the individual percent antagonism values ($n \geq 6$) were ranked to give the median and 95% confidence limits[4].

Results and discussion. Mecamylamine was the only antagonist tested which afforded complete blockade of the nicotine response (at twice the molar dose of nicotine; see table). It was also effective over a wide dose range (10 nmoles, i.e. one-tenth the molar dose of nicotine, and greater), but not over as broad a span as was hexamethonium. As little as 0.1 nmole of the latter still partially protected against the symptoms produced by 1000 times as much nicotine. Both agents appear to be much more effective than recently reported[5]. Decamethonium was much less active as an antagonist; like hexamethonium, it was a partial agonist at higher doses and produced a characteristic head tremor. Similarly, d-tubocurarine, a poor antagonist, could not be tested at higher doses without risk of inducing convulsions; appearance of these effects not only may explain the reduced efficacy and increased variance of the last 3 compounds at the highest doses reported, but also may have masked the antagonist properties of decamethonium and d-tubocurarine in the earlier study[2]. The vehicle used had no effect; mecamylamine was as effective when administered in distilled water as in saline, and saline itself did not alter the response to nicotine.

These results argue in favor of cholinergic involvement in the prostration-immobilization syndrome. Furthermore, the ganglionic blockers tested are more effective antagonists than the neuromuscular blockers (mecamylamine, though in the former class, also acts presynaptically[6]). In a recent study of a stereospecific nicotine binding site on rat brain membranes, Romano and Goldstein[7] concluded, on the basis of the agonists studied, that this site is similar to the peripheral ganglionic receptors, although they found that hexamethonium and mecamylamine inhibited nicotine binding much less than did d-tubocurarine and decamethonium, thus reversing the relative effectiveness we observe in vivo. Mecamylamine and hexamethonium displace nicotine only slightly from the intact and solubilized rat brain membrane preparations of Abood et al.[5].

Whether nicotine acts directly on cholinergic receptors or by relasing endogenous acetylcholine, elevating brain acetylcholine level should elicit similar responses. Intraventricular administration of physostigmine (10–50 nmoles) induced a wide range of effects, including hyperpnea, urination, defecation, staggering and head tremor, which are similar to a weak nicotine response, as well as salivation, gnawing and facial grooming, which are not observed with nicotine. Subsequent administration of acetylcholine chloride (0.5–100 nmoles) did not intensify the effects. In either case, tranquilization, weakness and sporadic gnawing and tremor lasted at least 20 min; the response to nicotine

The effect of selected antagonists on nicotine response[a]

Antagonist	Median percent antagonism at selected dose levels (nmoles) 0.1	0.5	1	5	10	50	100	200
Mecamylamine hydrochloride	–	–	0	–	50 (43–75)	75 (75–100)	69 (38–88)	100
Hexamethonium bromide	25 (0–86)	68 (50–75)	75 (71–87)	75 (50–100)	–	75 (71–100)	88 (75–100)	66 (58–71)
Decamethonium bromide	–	–	0 (0–25)	–	–	62 (38–75)	60 (0–86)	–
d-Tubocurarine	0	37 (0–83)	–	0	–	–	–	–

[a] Response evoked by 100 nmoles of nicotine; see 'Materials and methods' for definition of % antagonism.

usually disappears within 5 min. Prior administration of either atropine (50 nmoles) or hexamethonium (100 nmoles) delayed the appearance of tremor (induced by 50 nmoles of physostigmine) by 4–10 min, but neither agent reversed the effects of physostigmine when given after the esterase inhibitor. We conclude that nicotine's cholinergic action is necessary but not sufficient to explain the prostration syndrome.

1 The authors thank the Public Health Service for its generous support of this project (grant No. 1 F32 NS 06334-01 NEUB).
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Degeneration of retinal neuroblasts by chinoform-ferric chelate[1]

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Summary. The possible mechanism of neuropathic effect of chinoform was investigated using cultured retinal neuroblasts from chick embryos. Retinal neuroblasts completely degenerated by chinoform-ferric chelate within a day. This change, however, was not observed with free chinoform or ferric ion. *a*-Tocopherol had a potent protective effect on the toxicity of the chelate. From these results, it was concluded that the lipid peroxidation due to ferric ion chelated with chinoform incorporated into the membrane of nerve tissues is the most important step in induction of the neuropathy.

It was revealed by epidemiological research that subacute myelo-optico-neuropathy (SMON) was caused by massive doses of chinoform (5-chloro-7-iodo-8-quinolinol)[3,4]. Chinoform administered is known to be absorbed and incorporated into various organs including nerve tissues[5,6] and green-colored substance, appearing on the tongues and in the urine and feces of SMON patients, was identified as chinoform-ferric chelate[7]. In 1976, Yagi et al.[8] reported the effects of chinoform and chinoform-ferric chelate on an isolated sciatic nerve and found that chinoform-ferric chelate increased the lipid peroxides in the nerve sample, but free chinoform had no effect. They predicted that the lipid peroxidation initiated by chinoform-ferric chelate is the direct cause for the degeneration of the nerve tissues. To confirm this supposition, we intended to examine the toxicity of chinoform-ferric chelate on cultured retinal neuroblasts in comparison with free chinoform. The protection with an antioxidant, *a*-tocopherol, was also examined.

Materials and methods. Neural retinal cells were prepared by the method of Okada et al.[9]. Approximately 1.5×10^7 cells were inoculated into each Falcon plastic culture dish (35 mm in diameter) using 1.5 ml of a culture medium consisted of Eagle's minimum essential medium (MEM, NISSUI, Tokyo) supplemented with 8% fetal bovine serum (Gibco, New York), 0.3% L-glutamine and 0.14% sodium bicarbonate. The same lot of serum was used during the experiments. These culture dishes were incubated at 36.5 °C under an atmosphere of 5% CO_2-95% air. The culture medium was changed every 2 days for 5–6 days until the test. Chinoform and DL-*a*-tocopherol were sonicated in the medium to be emulsion. Chinoform-ferric chelate was prepared by sonicating the suspension of chinoform and $FeCl_3$ (molar ratio of chinoform to ferric ion, 10:3) in the medium. Morphological observation of the cells was made using an inverted phase contrast microscope (Zeiss, Invertoscope D).

To check the cell degeneration, dye exclusion test was carried out. After incubation of the cells with various reagents for a definite period, 0.2 ml of 0.5% erythrosine B in Ca^{2+}- and Mg^{2+}-free Hanks' solution (CMF) was added to a culture dish. 5 min later, the dish was fully washed with CMF 8–10 times. The cells were dissolved in 2.5 ml of 0.5 M KOH, and erythrosine B incorporated into the denatured cells was estimated by measuring the absorbance at 529 nm.

Results. Within 1–2 days after inoculation of cells, aggregates of cells adhered to culture dish. Thereafter, cells in aggregates started to spread. Aggregates were often interconnected with long axonal processes. At about 5 days, a sheet of flatened epithelial cells was formed, upon which small neuroblasts with axonal processes were superimposed (fig. 1, A). Epithelial cells were very thin, well-spread, transparent and poorly refractile under phase optics. On the other hand, neuroblasts were highly refractile due to their dense cytoplasm. Therefore, it was easy to distinguish neuroblasts from epithelial cells[9]. When 50 µM of chinoform-ferric chelate was added to neural retinal cells, degenerated neuroblasts were not observed until 3 h of incubation, and neuroblasts stained with erythrosine B were not detected. Some neuroblasts degenerated at 5 h (fig. 1, B), and at that time a slight increase of erythrosine B incorporation was observed (fig. 2). Then the incorporation of the dye into degenerated neuroblasts increased rapidly and the maximum incorporation was observed at 10 h after the addition of 50 µM of the chelate (fig. 2). These degenerated neuroblasts began to detach from a sheet of epithelial cells during further cultivation of these cells under the same condition, and cell debris floating in culture medium could be seen. Since the detached cells were removed from the dish by washing, incorporation of erythrosine B was decreased gradually from 10 to 24 h (fig. 2). Some degenerated cells still attached to epithelial cell sheet were