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Effects of ganglion blocking agents on nicotine extensor convulsions and lethality in mice

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1. The ganglion blocking agents, chlorisondamine, pentamethonium, mecamylamine, decamethonium and hexamethonium all block nicotine extensor convulsions when administered intraventricularly in mice. Tetraethylammonium was inactive.

2. For the intraventricular route, there is a relationship between ganglionic blocking potency and blocking of nicotine extensor convulsions. Indirect evidence suggests that the site(s) of action of nicotine extensor convulsions and lethality is central in origin and associated with brain areas near the ventricles.

3. When ganglion blocking agents are given orally, subcutaneously or intravenously varying degrees of protection can be observed probably depending on factors such as whether or not the drugs cross the blood-brain barrier, absorption, etc., and the effectiveness in protecting mice from nicotine is not related to ganglionic blocking potency.

4. Atropine and morphine given intraventricularly or subcutaneously did not protect mice from the LD95 of nicotine. Chlorpromazine gave very erratic results and phenobarbitone was effective subcutaneously and to a lesser extent intraventricularly.

Several reports have appeared in the literature regarding the effects of ganglion blocking drugs on the convulsant properties of nicotine (Tripod, 1949 ; Laurence & Stacey, 1952 ; Stone, Meckelnburg & Torchiana, 1956, 1958). Most investigators (see review of Silvette, Hoff, Larson and Haag, 1962) are in general agreement that the site(s) and mechanism(s) of action of nicotine are central although none has been clearly demonstrated. It was our feeling that much more information would be gained if the ganglion blocking drugs were given directly into the central nervous system rather than peripherally as reported in all the other studies. The method of Haley & McCormick (1957) in which unanaesthetized mice are injected intraventricularly seemed ideally suited for demonstrating possible central site(s) of action of nicotine and the ganglion blocking drugs. Because Stone, Meckelnburg & Torchiana (1958) showed that ganglion blocking agents antagonized the extensor response and death more readily than the clonic convulsions this study focused on nicotine extensor convulsions.

Methods

Fifteen male ICR mice per dose in the weight range 18–24 g were injected subcutaneously, intravenously, orally or intraventricularly (intracerebrally) with one of the ganglion blocking agents and at various times with a convulsant intravenous dose of nicotine. Appropriate controls were also tested. All doses except the intraventricular were calculated as base and geometrically spaced (2x) and given in a total volume of 0·1 ml./10 g body weight. For the intraventricular route of administration, the method of Haley & McCormick (1957) was used with the following additional modifications. A short piece of polyethylene tubing, 1·09 mm outside diameter, was placed over the shaft of a 27 gauge hypodermic needle so that a 3·5 mm portion of the front of the needle was exposed. The tube allowed only the exposed end of the needle to penetrate to the proper depth into the ventricles. A Hamilton microlitre (0·05 ml.) syringe was used to inject a total volume of 0·02 ml. per brain as base. In preliminary experiments, diluted (1 : 10) India ink was injected and the brains were removed, sectioned and examined to verify the site of injection.

A LD95 for nicotine (1·33 mg/kg) was determined by means of the dose-response curve and was injected over a 5 sec interval into a tail vein. The LD50 for nicotine was 0·58 mg/kg, the confidence limits were 0·49–0·79 and the slope of the dose-response curve was calculated to be 1·61. If an animal underwent a tonic-extensor convulsion it always died within 3 min. Otherwise, it survived for at least 24 hr. The latter observation period was used to determine the percentage mortality. If no protection was evident 2 hr after pretreatment with ganglion blocking agents, then a 30 min pretreatment test was also done in order to take into consideration possible different onsets of action or duration of action. Dose ranges for all the drugs tested are shown in Tables 1 and 2. All ED50s or LD50s were determined by the method of Litchfield & Wilcoxon (1949).

The drugs used and sources were as follows: atropine sulphate, Mallinckdrodt Chemical Co., St. Louis, Missouri ; chlorisondamine chloride, courtesy of Ciba Pharmaceutical Co., Summit, New Jersey ; chlorpromazine hydrochloride, courtesy of Smith, Kline and French Laboratories, Philadelphia, Pennsylvania ; decamethonium iodide, courtesy of Allen and Hanburys Ltd., Bayer Co., London ; hexamethonium bromide, courtesy of Chemicals Procurement Labs., Inc., College Point, New York ; mecamylamine hydrochloride, courtesy of Merck and Co., Inc., West Point, Pennsylvania ; morphine sulphate, Merck and Co., Inc. ; (−)-nicotine (+)-ditartrate, synthesized in this laboratory ; pentamethonium bromide, courtesy of Burroughs Wellcome and Co., Tuckahoe, New York ; phenobarbitone sodium, Merck and Co., and tetraethylammonium bromide, Eastman Kodak and Co., Rochester, New York.

Results

The results are summarized in Tables 1 and 2. Whenever possible, ED50s, 95% confidence limits, and slopes of regression lines were determined ; otherwise, the results are expressed as % of mice protected. Although no detailed overt behavioural studies were planned various changes were recorded whenever noted and are included in the tables.

When given intraventricularly either 30 min or 2 hr before the nicotine, all the ganglion blocking agents except tetraethylammonium were effective in preventing

TABLE 1. *Effects of ganglion blocking agents by different routes of administration on nicotine extensor convulsions*

Drug	Route of administration	Pretreatment time (min)	Dose range	ED50 (confidence limits) and slope	Overt changes drug alone
Chlorisondamine	i.c.	120	0·024–50 μg brain	0·18 (0·11–0·30) μg/brain $S=3·31$	Some tail vasodilation and some stimulation
	i.v.	120	0·031–8 mg/kg	1·75 (1·17–2·53) mg/kg $S=2·71$	——
	s.c.	120	1–16 mg/kg	1·68 (1·12–2·52) mg/kg $S=2·73$	Vasodilation of tail veins, some eye ptosis. Straub tail at 16 mg/kg
	p.o.	120	1–64 mg/kg	34 (23·5–40·3) mg/kg $S=2·49$	Vasodilation of tail veins, stimulation
Pentamethonium	i.c.	30	0·048–50 μg/brain	1·75 (1·00–3·06) μg/brain $S=5·83$	Depression followed by stimulation 50 μg lethal to 1/15
	s.c.	30	0·5–32 mg/kg	7·5 (5·17–10·88) mg/kg $S=2·49$	Slight stimulation
	s.c.	120	0·5–32 mg/kg	6.7% protection at 8 mg/kg, 20% protection at 16 mg/kg and 20% protection at 32 mg/kg	Slight stimulation
Mecamylamine	i.c.	120	0·78–25 μg/brain	10·8 (6·96–16·7) μg/brain $S=3·49$	——
	i.v.	120	0·031–16 mg/kg	0·45 (0·31–0·75 mg/kg $S=2·89$	Lethal to 13/15 at 16 mg/kg clonic convulsion
	p.o.	120	0·0078–32 mg/kg	0·33 (0·248–0·439) mg/kg $S=1·76$	——
	s.c.	120	0·0078–64 mg/kg	0·430 (0·29–0·62 mg/kg $S=2·36$	——
Hexamethonium	i.c.	120	3·12–200 μg/brain	14·5 (9·60–21·90) μg/brain $S=3·3$	Stimulation 5–10 min after medication
	i.v.	120	1–64 mg/kg	6·7% protection at 2 mg/kg	15/15 dead at 64 mg/kg
	s.c.	120	2–64 mg/kg	14·3% protection at 64 mg/kg	Lethal 1/15 at 64 mg/kg
	p.o.	120	2–128 mg/kg	13·3% protection at 32 and 60% protection at 64 mg/kg	——
	i.v.	30	0·5–64 mg/kg	20% protection at 8 and 73·3% protection at 16 mg/kg	Lethal 1/15 at 32 and 12/15 tremors, laboured breathing at 64 mg/kg

TABLE 1—*continued*

Drug	Route of administration	Pretreatment time (min)	Dose range	ED50 (confidence limits) and slope	Overt changes drug alone
Decamethonium	i.c.	120	6·25–200 μg brain	6·7% protection at 6·25 μg/brain	Lethal at 100 and 200 μg/brain hyperexcitability
	i.v.	120	0·031–2 mg/kg	No protection	Lethal 13/15 at 1 mg/kg and 15/15 at 2 mg/kg
	s.c.	120	1–8 mg/kg	No protection	Lethal at 2, 4, and 8 mg/kg tremors, laboured breathing
	i.c.	30	3·12–100 μg/brain	26·5 (19·2–36·6) μg/brain $S=2·24$	Hyperexcitability, tremors, ataxia at 25, 50, 100 μg/brain lethal to 8/15 at 100 μg/brain
	i.v.	30	0·015625–2 mg/kg	No protection	Lethal to 13/15 at 1 and 15/15 at 2 mg/kg, tremors, laboured breathing
	s.c.	30	0·31–4 mg/kg	No protection	Lethal to 5/15 at 4 mg/kg
	p.o.	30	1–128 mg/kg	No protection	Lethal to 2/15 at 128 mg/kg
Tetraethylammonium	i.c.	120	0·78–50 μg/brain	No protection	Lethal at 50 μg/brain to 4/15 mice, tremors, excitability
	i.v.	120	1–32 mg/kg	No protection	Lethal at 16 mg/kg to 1/15 mice, lethal at 32 mg/kg to 14/15 mice
	s.c.	120	1–64 mg/kg	No protection	——
	p.o.	120	1–64 mg/kg	No protection	——
	i.c.	30	0·78–50 μg/brain	No protection	Stimulation hyperexcitable, convulsions, lethal at 50 μg/brain 3/15 mice
	i.v.	30	0·25–32 mg/kg	No protection	Lethal to 8/15 at 32 mg/kg
	s.c.	30	1–64 mg/kg	No protection	——
	p.o.	30	1–64 mg/kg	No protection	——

TABLE 2. *Effects of selected drugs with actions in the central nervous system on nicotine extensor convulsions*

Drug	Route of administration	Pretreatment time (min)	Dose range	ED50 (confidence limits) and slope	Overt changes drug alone
Atropine	i.c.	120	3·12–200 μg/brain	No protection	Stimulation, running biting Lethal at 50, 100 and 200 μg/brain per mouse
	s.c.	120	1–128 mg/kg	13·3% protection at 32 mg/kg	Stimulation, running jumping
	i.c.	30	3·12–200 μg/brain	6·7% protection at 25 μg/brain	Stimulation, running biting. Lethal at 50, 100 and 200 μg/brain per mouse
	s.c.	30	1–128 mg/kg	6·7% protection at 1 mg/kg. 20% protection at 32 mg/kg and 6·7% protection at 128 mg/kg	Stimulation, running
Chlorpromazine	i.c.	120	3·12–200 μg/brain	21·4% protection at 50 μg/brain, 27·3% protection at 100 μg/brain and 60% protection at 200 μg/brain	Depression, ptosis some convulsions, lethal at 50, 100 and 200 μg/brain
	s.c.	120	1–128 mg/kg	20% protection at 4 mg/kg, 46·7% ptotection at 8 mg/kg 40% protection at 16 mg/kg, 73·3% protection at 32 mg/kg, 46·7% protection at 64 mg/kg and 60% protection at 128 mg/kg	Depression, ptosis
Morphine	i.c.	120	1·56–200 μg/brain	No protection	Stimulation, running, some Straub tail, lethal at 100 and 200 μg/brain
	s.c.	120	1–128 mg/kg	No protection	Stimulation, running, Straub tail
	i.c.	30	1·56–200 μg/brain	No protection	Same as i.c. above
	s.c.	30	1–128 mg/kg	No protection	Same as s.c. above
Phenobarbitone	i.c.	120	3·12–200 μg/brain	No protection	Stimulation, running, hyperexcitable.
	s.c.	120	0·5–64 mg/kg	13 (9·2–18·5) mg/kg, $S=1·98$	Stimulation, running, biting
	i.c.	30	12·5–800 μg/brain	40% protection at 200 μg 90% protection of survivors at 400 μg/brain	Stimulation, running. Loss of righting reflex at 400 and and 800 μg/brain. Convulsions and death lethal to 5/15 at 400 μg/brain and 14/15 at 800 μg/brain

nicotine extensor convulsions and death. Chlorisondamine was the most active compound tested ; an ED50 of 0·18 μg/brain was calculated. Pentamethonium was effective in protecting 50% of the animals at 1·75 μg/brain, mecamylamine at 10·8 μg/brain, hexamethonium at 14·5 μg/brain and decamethonium at 26·5 μg/brain 30 min before nicotine. The latter drug was inactive 2 hr after injection possibly because of short duration of action. Because of limited supplies pentamethonium was not tested intraventricularly at 2 hr.

Given orally, subcutaneously or intravenously, mecamylamine was the most active, the oral route being the most active. Chlorisondamine was much more active subcutaneously (ED50 1·68 mg/kg) and intravenously (ED50 1·75 mg/kg) than orally (ED50 34 mg/kg). Pentamethonium was only tested subcutaneously for the reason cited above and the 30 min ED50 was found to be 7·5 mg/kg. The drug was not effective when given 2 hr before the nicotine challenge dose. Hexamethonium produced only weak protection when given by all the routes after 30 min or 2 hr, except for 30 min after the intravenous dose in which 73% protection was evident at 16 mg/kg. Decamethonium and tetraethylammonium did not block nicotine extensor convulsions after 30 min or 2 hr by any route.

Table 2 shows the results obtained when various classes of drugs with known central nervous system properties were tested. Atropine, and morphine were inactive by both the intraventricular and subcutaneous routes and chlorpromazine gave an erratic dose response effect, whereas phenobarbitone was much more active subcutaneously than it was intraventricularly. The subcutaneous ED50 was 13 mg/kg.

Discussion

It is evident that the ganglion blocking agents are effective in protecting mice from nicotine extensor convulsions and death for as long as 24 hr especially when given intraventricularly. Tetraethylammonium was the only drug tested not found active and it is interesting that Stone, Meckelnburg & Torchiana (1958) felt that the drug did not demonstrate peripheral ganglionic blocking activity in mice as estimated by the mouse mydriasis test (see Table 3). These same authors also reported that there was no correlation between antinicotine activity and ganglionic potency when the ganglion blocking agents, were given intraperitoneally. For the oral, subcutaneous and intravenous routes our data are in agreement with the observation reported by these authors ; however, a relationship can be shown for the intraventricular route (Table 3).

TABLE 3. *Correlation of intraventricular potencies of ganglion blocking agents in protecting mice from extensor convulsant and lethal doses of nicotine with ganglionic potencies as measured by the mouse pupil dilatation test*

Drug	Intraventricular ED50 μg/brain	Mouse pupil dilatation test* (mg/kg i.p.)
Chlorisondamine	0·18 (0·11-0·30)	0·05 (0·045-0·055
Pentolinium	Not determined	0·33 (0·27-0·040)
Pentamethonium	1·75 (1·00–3·06)	Not reported
Mecamylamine	10·8 (6·96–16·7)	1·35 (1·22–1·50)
Hexamethonium	15·5 (9·0–21·3)	10·2 (8·0–13·0)
Decamethonium	26·5 (19·2–36·6)	Not reported
Tetraethylammonium	>50	>32

* Taken from Stone, Meckelnburg & Torchiana (1958).

Thus, it appears that the ganglion blocking agents and nicotine are acting centrally and that possibly the site(s) of action may be associated with the ventricular areas since the former probably do not penetrate brain tissue. It is unlikely that leakage of drug followed by absorption from the subarachnoid space into the systemic circulation could take place and account for the effects we noticed, since some of the ganglion blocking drugs were completely inactive when given peripherally even in massive doses and since those which were active peripherally required much larger doses than could have leaked into the systemic circulation. It is known that local application of nicotine to the motor cortex produces clonic convulsions in dogs (Rizzolo, 1929) and it has been suggested that nicotine convulsions might be due to anoxia resulting from respiratory arrest or to stimulation of the carotid body (Lendle & Ruppert, 1942) but the convulsions produced in this study are not clonic but extensor and they always occur before respiratory arrest, so that anoxia cannot be the cause. The intraventricular studies rule out the possibility of carotid body stimulation as a cause.

Our data is in agreement with that of Stone, Meckelnburg & Torchiana (1958) with regard to the inability of tetraethylammonium to block nicotine extensor convulsions and in disagreement with that of Tripod (1949) who reported that this ganglion blocking agent was active. Stone, Meckelnburg & Torchiana (1958) also reported that hexamethonium was effective versus nicotine when given intraperitoneally but we could not demonstrate activity by the subcutaneous route, although the drug did demonstrate 73% protection when given intravenously at 30 min. It is possible that this drug is not well absorbed by the other routes. That drugs such as atropine and morphine when given intraventricularly and subcutaneously in large doses were unable to protect mice from a convulsant and lethal dose of nicotine suggests that the site of action of nicotine for producing extensor convulsions is rather specific and that the action of the ganglion blocking agents is also specific. Chlorpromazine gave incomplete and erratic protection and our data do not allow for much speculation.

That phenobarbitone was poorly active intraventricularly and more effective subcutaneously when given 2 hr before the nicotine challenge may be explained by suggesting that it diffused out of the brain into the systemic circulation during the pretreatment time and thereby reduced the concentration of the drug. This explanation is supported by the fact that 40% protection was obtained in the 30 min premedication study. Apparently, the initial concentration in the ventricles is near the lethal dose but quickly subsides as the drug diffuses out of the ventricles.

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