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CHEMICAL SYMPATHECTOMY BY GUANIDINIUM ADRENERGIC NEURON BLOCKING AGENTS*

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Abstract—Chronic administration of high doses of the adrenergic neuron blocking agent, guanethidine, to neonatal or adult rats produces destruction of the sympathetic nervous system (sympathectomy). Although the mechanism of cytotoxicity is not established, it has been proposed to be due to inhibition of oxidative phosphorylation, an activity of guanethidine which has been demonstrated *in vitro* in isolated mitochondria. In this study, 40 guanidinium adrenergic neuron blocking agents were examined for cytotoxic effects on sympathetic neurons when administered chronically to neonatal rats. The ability of many of the compounds to concentrate in sympathetic neurons was determined, using, in most cases, an assay based on the development of a phenanthrenequinone derivative. The ability of several of the compounds to inhibit oxidative phosphorylation in isolated rat liver mitochondria was also determined. The results show that only a few compounds, very similar in structure to guanethidine, are cytotoxic *in vivo*. Only compounds very similar to guanethidine accumulate to high concentrations in the neuronal cell bodies, and this may explain the lack of toxicity of some compounds. However, compounds were found, particularly Pharmacia 881/7, which accumulate in the cell body but are not cytotoxic. In isolated mitochondria, Pharmacia 881/7 was ten times more potent than guanethidine as an inhibitor of oxidative phosphorylation. Some compounds which were cytotoxic *in vivo* produced little or no inhibition of oxidative phosphorylation. These data indicate that inhibition of oxidative phosphorylation is not the mechanism by which guanethidine destroys sympathetic neurons.

The chronic administration of high doses of the guanidinium adrenergic neuron blocking agent, guanethidine, to newborn or adult rats produces destruction of the sympathetic nervous system [1–3]. The ability to produce chemical sympathectomy is not inherent in adrenergic neuron blocking activity, since the dose of guanethidine (Fig. 1; compound 1) required to produce sympathectomy is much higher than that required for neuronal blockade. In addition, other guanidinium blocking agents, including clinically used agents such as debrisoquin, bethanidine and guanoxan, do not destroy the neurons *in vivo* [4, 5].

An obvious possibility to explain the difference in cytotoxicity of the compounds *in vivo* would be differences in the accumulation of the drugs in the cell bodies of the affected neurons, since inhibition of the accumulation of guanethidine in the ganglia by desmethylimipramine prevents the cytotoxic effects [6]. The ability of only a few compounds to accumulate in sympathetic ganglia has been assessed [7], and hence any general conclusions are not possible.

The mechanism by which the cytotoxic effect is produced is not known. Interest in this question is increased by the demonstration that concomitant administration of nerve growth factor can prevent neuronal cell death in neonatal rats [5]. It has been suggested [5, 8] that the ability of guanethidine to inhibit oxidative phosphorylation [9] underlies its cytotoxic effect. Guanethidine accumulates to a concentration of approximately 0.5 mM in sympathetic neurons [6], and mitochondria appear to be the first organelles adversely affected [8]. If this is indeed the mechanism of cytotoxicity, it would be predicted that the product of potency as an inhibitor of oxidative phosphorylation and accumulation in sympathetic ganglia should predict the cytotoxic activity of these compounds.

The objective of the present study was to determine the ability of a large series of guanidinium adrenergic neuron blocking agents to destroy the sympathetic nervous system of neonatal rats when injected chronically at high doses. The cytotoxicity *in vivo* was related to the accumulation or lack thereof in sympathetic ganglia. In order to do this, we developed a more sensitive assay to measure many of these compounds in tissue. We then selected compounds to examine as inhibitors of oxidative phosphorylation in isolated mitochondria.

MATERIALS AND METHODS

Compounds studied. Hundreds of guanidinium or guanidinium-like compounds have been synthesized and studied as adrenergic neuron blocking agents (reviewed in Ref. 10). Compounds were obtained from various manufacturers (Hoffmann-La Roche, Inc., Nutley, NJ; Ciba Pharmaceutical Co., Summit, NJ; Pfizer, Inc., Groton, CT; Pharmacia, Hillerod, Denmark; The Upjohn Co., Kalamazoo, MI; Abbott Laboratories, Chicago, IL; and Wellcome Laboratories, Beckingham, Kent, U.K.). We are particularly indebted to Dr. Ronald Kuntzman and colleagues at Hoffmann-La Roche who supplied most of the compounds. The

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structures of 41 compounds are shown in Figs. 1 and 2; all were studied (except compound 7, guanacline, which was not available). All the compounds were examined for impurities by descending paper chromatography in two solvent systems [3% NH_4OH–isobutanol (1:3) and butanol–acetic acid–water (60:14:25)], followed by visualization in iodine vapor. No significant impurity was noted in any of the compounds.

Animal treatments. All drugs were administered at a dose of 50 mg/kg/day of the available salt in aqueous solution. Neonatal Sprague–Dawley rats were injected, starting when the animals were 8 days old, 5 days/week for 2 weeks. Animals were killed on the day after the third injection for analysis of accumulation in the superior cervical ganglia (SCG). Animals were killed 7 days and 14 days after starting treatment and SCG were excised and processed for light microscopic examination.

Light microscopy. Upon removal from the animal, SCG were fixed in 2.5% buffered glutaraldehyde, dehydrated, embedded in paraffin, cut in 7-μM sections and stained with toluidine blue.

Analysis in tissue. SCG were frozen upon dissection from the animals. Ganglia were homogenized and extracted essentially as described by Schanker and Morrison [11]. Ganglia (3 pair/analysis) were homogenized in 0.35 ml of 0.1 N HCl, and 0.3 ml of this was transferred to a 16 × 150 mm tube containing 35 μl of 5 N NaOH and 8.0 ml of $CHCl_3$. After mixing and centrifugation, the aqueous phase was aspirated off, and 1.0 ml of 0.1 N NaOH was added, mixed and centrifuged; the aqueous phase was discarded again. The $CHCl_3$ was extracted with 1 ml of 0.2 N HCl, and 0.75 ml of the aqueous phase was transferred to a small test tube. Fluorescence was developed as either the ninhydrin derivative in alkaline solution, as described by Schanker and Morrison [11], or as a phenanthrenequinone (PTQ) derivative. The PTQ assay is based upon the procedure of Yamada and Itano [12] developed for the analysis of arginine and other nonsubstituted guanidines. To 0.75 ml of the extracted aqueous phase were added, in order, 0.14 ml of 5 N NaOH and 0.06 ml of PTQ (0.1 mg/ml in 95% ethanol, freshly prepared), and this mixture was allowed to stand overnight at 4°; 0.3 ml of 5 N HCl was then added, and the fluorescence was determined on an Aminco–Bowman spectrophotofluorometer at an excitation wavelength of 325 nm and emission 395 nm (uncorrected). The fluorescence is linear through a wide concentration range and stable for several hours. In all cases, blanks consisted of extracted ganglia from untreated animals.

Oxidative phosphorylation in isolated rat liver mitochondria. Isolated mitochondria were obtained from the livers of female Sprague–Dawley rats by a modification of the method of Chance and Hagihara [13]. Rats were decapitated and exsanguinated; the liver was removed and placed in iced 0.33 M sucrose for 5 min. The livers were minced, weighed, and homogenized in a glass–Teflon Potter–Elvehjem homogenizer in 10 vol.

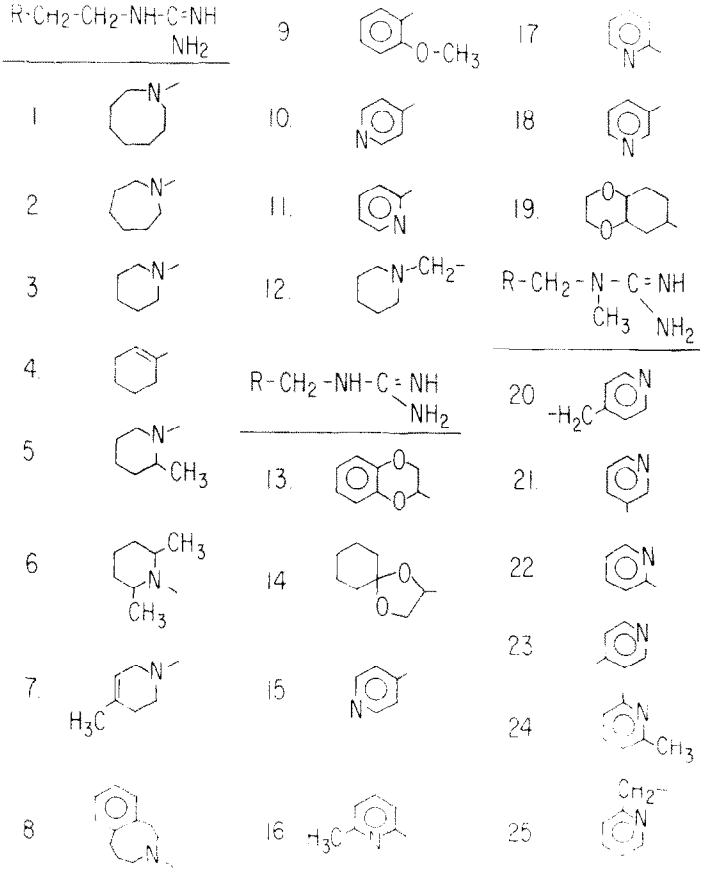

Fig. 1. Structures of compounds examined. Compounds referred to in text by number shown.

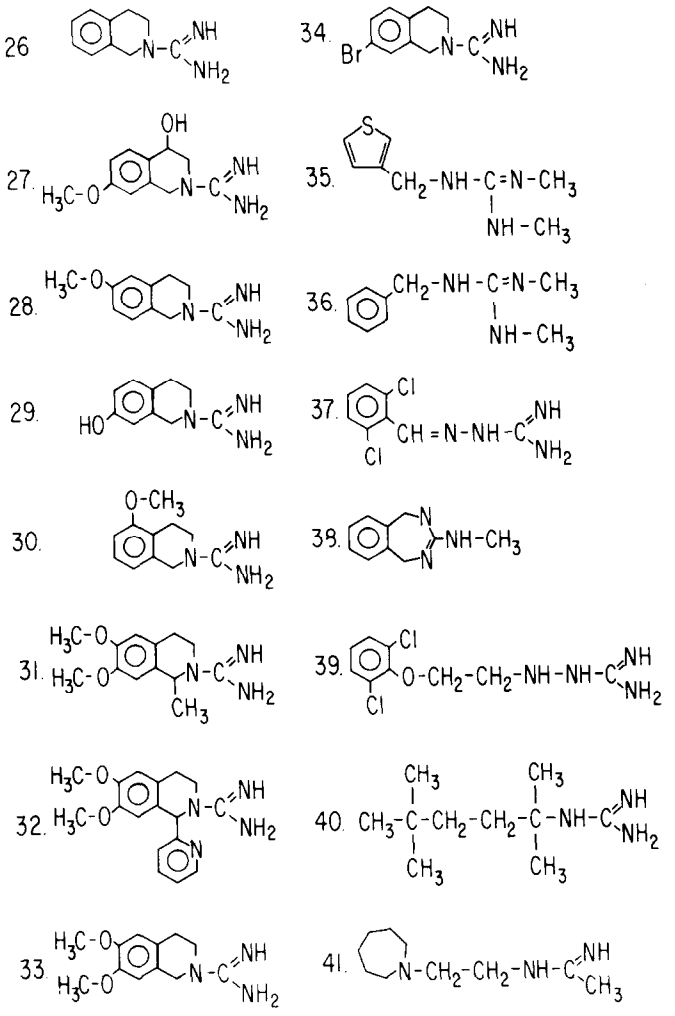

Fig. 2. Structures of compounds examined. Compounds referred to in text by number shown.

of cold SMEB (75 mM sucrose, 225 mM mannitol, 0.2 mM EDTA, 2 mM MOPS buffer, pH 7.4, 3 mg/ml of crystalline fatty acid free BSA). Cellular debris and nuclei were removed by centrifuging for 5 min at 1000 *g* in a Lourdes refrigerated centrifuge with a 9RA head. The mitochondria were collected by centrifuging at 12,000 *g* for 10 min, washed twice with centrifugation for 5 min at 8000 *g*, and resuspended in SMEB to about 25 mg protein/ml.

Inhibition of mitochondrial respiration and phosphorylation was studied with a Beckman macro oxygen electrode in a 2.5-ml cuvette closed to the air except for a small aperture for insertion of microsyringe needles. The quantity of mitochondrial preparation used (25 μl) yielded a rate of respiration which would have exhausted the oxygen in 10–15 min, if sufficient ADP were provided. The incubation medium consisted of 75 mM sucrose, 225 mM mannitol, 10 mM phosphate, pH 7.4, 10 mM Tris–HCl, pH 7.4, 3 mM $MgCl_2$, 0.2 mM EDTA, and 1 mg/ml of BSA. Substrates used were glutamate (8 mM) or succinate (8 mM). Microliter quantities of ADP and 2.4-dinitrophenol (DNP) were added at appropriate times. The consumption of O_2 at 25° was recorded with a Varian or a Leeds and Northrup recorder, with the rate of oxygen consumption determined from the slopes of the lines.

RESULTS

Comparison of assay procedures. The concentration of guanethidine in tissues has been studied using the ninhydrin fluorometric method developed by Schanker and Morrison [11]. However, the use of this method to analyze accumulation in small amounts of tissue, such as in ganglia, tests the sensitivity of the procedure. Therefore, we developed a method based on the formation of a phenanthrenequinone derivative. Using the method described by Yamada and Itano [12] for the assay of arginine as a starting point, we systematically varied conditions to produce optimum fluorescence; those conditions are described in Materials and Methods. The primary advantages over the ninhydrin assay are sensitivity and the stability of the fluorescent derivative. A systematic examination of the two methods of producing fluorescent derivatives of the compounds studied is shown in Table 1. Also shown is the recovery of the compound through the extraction procedure.

Table 1. Comparison of ninhydrin and PTQ fluorescence of guanidinium adrenergic neuron blocking agents and recovery through the extraction procedure

Compound†	Sample/blank ratio* Ninhydrin	PTQ	% Recovery
1	4.7	22	80
2	ND‡	16.6	37
3	5	19	34
4	4.7	7.5	33
5	2.8	23.6	44
6	3	18	37
7	Not available		
8	2.8	17.3	67
9	4	8	65
10	2.8	14	11
11	ND	6.4	0
12	2.9	9.7	40
13	2.1	13.8	71
14	1	3	0
15	4	33	3
16	1	24	23
17	4	27	7
18	0.6	32	0
19	2.8	23	26
20	18.1	1.4	15
21	20	1.3	13
22	24.5	1.5	43
23	17.1	1	10
24	16.5	1	57
25	13	1	0
26	17	1	74
27	15	1	ND
28	11	1	ND
29	9	1	0
30	ND	ND	ND
31	11	1	ND
32	14	1	ND
33	10.2	1	0
34	13	1	45
35	1	1	ND
36	1	1	ND
37	1	1	ND
38	1	1	ND
39	1	1	ND
40	2.8	1	0
41	1	1	ND

* Solutions of the compound (300 ng/0.75 ml) in 0.2 N HCl were prepared, and the fluorescence was developed by either ninhydrin or PTQ, as described in Materials and Methods. In most cases, the reported values are from a single experiment.

† Structures shown in Figs. 1 and 2.

‡ Not determined.

PTQ produced a much higher sample to blank ratio than did ninhydrin for most of the compounds (1–19). For example, the ratio is five times higher in the case of guanethidine (compound 1). These ratios of sensitivity are applicable to actual assay conditions, since tissue blanks (i.e. extracted ganglia from untreated animals) are twice as high as solvent blanks with both ninhydrin and PTQ. Compounds for which ninhydrin is superior are the isoquinoline carboxamide compounds (26–34) such as debrisoquin (compound 26) and the compounds (20–25) in which a methyl group is attached to the interior guanidine nitrogen. Some compounds (35–41) did not produce fluorescent derivatives with either ninhydrin or PTQ.

Effect of chronic treatment on sympathetic neurons. The compounds shown in Figs. 1 and 2 were administered at a dose of 50 mg/kg/day, s.c., 5 days/week for 2 weeks, and possible cytotoxicity was assessed by light microscopic examination of SCG after 1 and 2 weeks of treatment. The only exceptions were compounds 21, 23, 28, 32 and 40, which were too toxic for study at this dose. Compounds 28 and 32 were studied at 20 mg/kg. Only four compounds (1, 2, 3 and 5) were found to have an adverse effect on sympathetic neurons. Compound 7 (guanacline) was not available, but previous studies [4] have shown it to produce sympathectomy. Compounds 1, 2, 3 and 5 caused massive destruction, which was apparent at the end of 1 week. Most of the neurons had disappeared or were clearly degenerating by this time, and there was marked small cell infiltration of the ganglia. Even after 2 weeks of treatment, ganglia from animals treated with the other compounds appeared normal upon light microscopic examination.

Accumulation of guanethidine and other compounds in sympathetic ganglia. Initial studies were carried out to determine the accumulation characteristics of guanethidine in the SCG of neonatal rats. The time course of accumulation of guanethidine is shown in Fig. 3. Guanethidine (measured 24 hr after the last injection) reaches maximal levels in the SCG after two injections and remains at a constant level through day 5. The accumulation of guanethidine 3 days after starting treatment at various doses of guanethidine is shown in Fig. 4. Note that the concentration increases as a function of dose up to 30 mg/kg/day. Consistent with previous studies [1] in adult rats, it was found that doses of 5 and 10 mg/kg/day of guanethidine do not produce destruction of the ganglia, whereas doses of 20 mg/kg or greater produce marked cell death. Hence, a concentration of guanethidine of approximately 0.3 nmole/pair or greater appears to be required for cytotoxicity.

Based upon the data in Table 1 (formation of fluorescent derivatives and reasonable recovery ratios), seventeen compounds were chosen for the study of accumulation in SCG. All compounds were administered at a

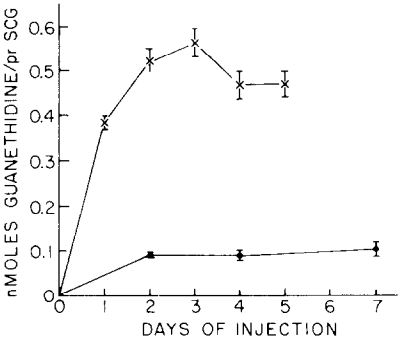

Fig. 3. Time course of accumulation of guanethidine in superior cervical ganglia of neonatal rats. Animals were given 50 mg/kg/day (×——×) or 5 mg/kg/day (●——●), s.c., of guanethidine sulfate. Animals were killed on the day after the last injection. Superior cervical ganglia were excised and assayed for guanethidine by the PTQ assay described in Materials and Methods. Each value is the mean ± S.E.M. of three to four samples containing three pair of ganglia.

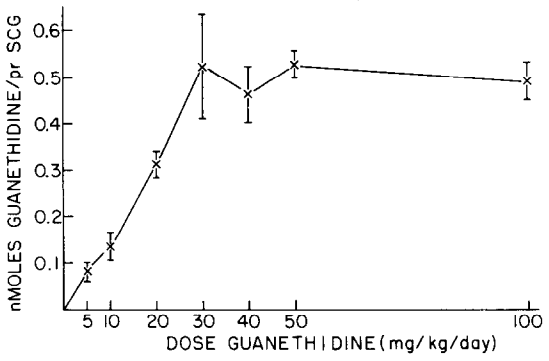

Fig. 4. Accumulation of guanethidine in superior cervical ganglia of neonatal rats as a function of dose. Drug was administered as a single daily injection for 3 days. Animals were killed 24 hr after the last injection and ganglia were analyzed for guanethidine content by PTQ assay, as described in Materials and Methods. Each value is the mean ± S.E.M. of three to six samples containing three pair of ganglia.

dose of 50 mg/kg/day for 3 days and the animals killed 24 hr after the last injection. The data in Table 2 show that all compounds (1, 2, 3 and 5) previously found to be cytotoxic to the neuron accumulated to a concentration of greater than 0.5 nmole/pair of ganglia. Indeed, of the cytotoxic compounds guanethidine reached the lowest concentration. The majority of non-cytotoxic compounds accumulated to a very small extent in the ganglia. Two exceptions are compound 8 (Pharmacia 881/7) and compound 12, which are structurally similar to guanethidine and accumulate to concentrations 70 and 40% of that of guanethidine respectively.

Effects of compounds on oxidative phosphorylation. If inhibition of oxidative phosphorylation is the mechanism by which drugs produce death of sympathetic neurons, it would be predicted that the drugs which destroy the neurons (compounds 1, 2, 3 and 5) would be active and that the compounds which accumulate but are not cytotoxic (particularly compounds 8 and 12) would be far less active inhibitors of oxidative phosphorylation. In order to test this prediction, eight of the compounds were studied as inhibitors of oxidative phosphorylation in isolated rat liver mitochondria. The effects of the compounds on state 3 respiration using glutamate as substrate are shown in Table 3. Also shown are the accumulation in ganglia (from Table 2) and cytotoxic activity, to facilitate comparison. All of the compounds which inhibited state 3 respiration showed signs of progressively increasing inhibition with time of exposure. The inhibition was checked at 5, 10 and 15 min in all cases and occasionally at 20 min. The data in Table 3 were taken from 15-min exposures. The time dependent nature of the inhibition produced by compound 26 (debrisoquin) is shown in Fig. 5. The data in Table 3 show that the compounds vary widely in potency as inhibitors of state 3 respiration, using glutamate as substrate. Compounds 8 and 26 (Pharmacia 881/7 and debrisoquin) are about 10 times as potent as guanethidine. The results obtained with debrisoquin and guanethidine are consistent with those reported previously [9]. The somewhat lower ED_{50} values reported here for compounds 1 and 26 are the result of measuring inhibition 15 min after addition of the test compound, rather than 5 min as in the previous study [9]. Reducing the size of the heterocyclic ring (compounds 2 and 3) greatly reduced activity from that produced by guanethidine. The action of all of the compounds would appear to be primarily inhibition at the NADH dehydrogenase level, with glutamate as substrate. Most of the compounds in Table 3 had little effect on respiration with succinate as substrate at concentrations producing 50 per cent inhibition of glutamate-supported respiration. The distinct exception was compound 26, which caused 50 per cent inhibition of a succinate oxidation at 100 μM.

Because Malmquist and Oates [9] and others [14, 15] have reported differences in the rate of onset of inhibition by guanidine derivatives under different conditions, we tested the degree of inhibition and the rate of onset with each compound added before the mitochondria had been exposed to ADP (external ATP low) and

Table 2. Accumulation of guanidinium adrenergic neuron blocking agents in the superior cervical ganglia of neonatal rats *

Compound†	nmoles/pair	Compound	nmoles/pair
1	0.56 ± 0.03	12	0.22 ± 0.04
2	0.94 ± 0.11	13	0.11 ± 0.01
3	1.05 ± 0.14	16	0.06 ± 0.01
4	0	19	0.12 ± 0.03
5	1.40 ± 0.09	20	0
6	0.15 ± 0.01	22	0.08 ± 0.01
8	0.39 ± 0.01	24	0
9	0	26	0.03 ± 0.01
		34	0

* Neonatal rats (7-days-old) were treated with the various drugs (50 mg/kg/day) for 3 days. Animals were killed 24 hr after the last injection and assayed as described in Materials and Methods. Each value represents the mean ± S.E.M. of at least three samples (each sample containing three pairs of ganglia).

† Structure shown in Figs. 1 and 2.

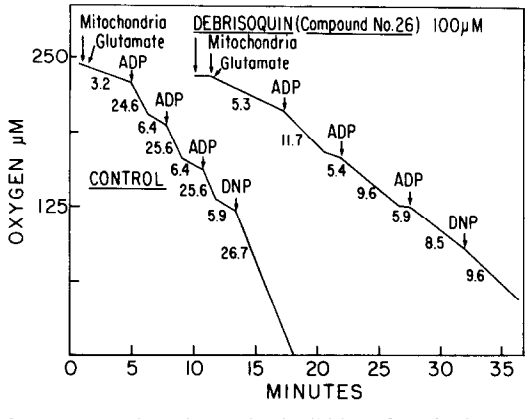

Fig. 5. Progressively increasing inhibition of respiration over 15 min by compound 26 (debrisoquin) at 100 μM. The numbers by each portion of the O_2 electrode tracing are the rates (nmoles of O_2 consumed/min/ml). The ADP/O ratios calculated from the extra O_2 consumption induced by ADP are about 2.6 for both the control and the debrisoquin tracings. Mitochondria = 1 mg protein/ml, glutamate 8 mM, ADP 96 μM and DNP 40 μM final concentration. The medium is described under Materials and Methods.

Table 3. Inhibition of mitochondrial respiration by guanidinium adrenergic neuron blocking agents

Compound*	Inhibition of State 3 respiration (ED_{50} in μM)	Concentration in ganglia†	Cytotoxicity
8	40	0.39	—
26	50	0.03	—
13	100	0.11	—
5	200	1.40	+
1	500	0.56	+
2	>1000	0.94	+
3	≫1000	1.05	+
12	≫1000	0.22	—

* Structures shown in Figs. 1 and 2.
† From Table 2.

after one ADP addition and a burst of oxygen consumption (higher external ATP concentration). No marked difference in inhibition by the compounds was noted.

When the guanidine derivatives were added during state 4 respiration after an ADP-induced burst of oxygen consumption, the concentration which produced a 50 per cent inhibition of state 3 rate had little or no uncoupling action. Slight uncoupling actions were seen at high concentrations of compounds 8 and 26. In all cases, concentrations producing complete inhibition of state 3 respiration caused no inhibition of state 4 respiration. All inhibitions were checked to see if they were released by uncoupling concentrations of 2, 4-dinitrophenol (DNP, 40 μM). State 3 rates inhibited by the drugs were not released when glutamate was the substrate. However, any fraction of state 3 respiration not inhibited by the guanidinium compounds was released. The state 3 inhibition by compound 26 when succinate was the substrate was completely released by DNP.

Indentification of compounds accumulating in the ganglia. Any conclusion reached relating measured amounts of guanidinium compound in ganglia, cytotoxicity, and inhibition of oxidative phosphorylation rests upon the assumption that the material measured in the ganglia is unchanged drug and not a metabolite. In order to test this assumption, chloroform extracts of ganglia from animals treated with guanethidine or Ph881/7 (compounds 1 and 8), as described in Table 2, were chromatographed on Eastman Cellulose Chromagrams, and the chromatograms were developed by spraying with a phenanthrenequinone spray reagent [12]. In a solvent system of butanol–acetic acid–water (4:1:1), the guanidinium compound extracted from the ganglia of guanethidine-treated animals had the same R_f (0.65) as guanethidine. Likewise, the compound extracted from the ganglia of Ph881/7-treated animals had the same R_f (0.71) as Ph881/7. The same result was obtained when extracts were chromatographed in ethanol–water (70:30), where the R_f values of guanethidine (0.12) and Ph881/7 (0.19) were the same as that of the extracted guanidinium compounds. In both solvent systems, only a single spot was seen in the tissue extracts. Similar extracts from untreated animals showed no phenanthrenequinone positive spots. Hence, it appears that unmetabolized drug is the only species accumulating in the ganglia of the treated animals.

DISCUSSION

The present study, involving 40 compounds, greatly expands the number of guanidinium adrenergic neuron blocking agents examined for cytotoxic effects on sympathetic neurons. Juul [4, 7] has studied the effects of ten compounds on adult rats, including eight of the compounds reported here (1, 8, 13, 26, 34, 36, 39, and 40). In that study only compound 1 (guanethidine) was found to be cytotoxic. Our results, obtained in neonates, are consistent with those results. The data suggest that there is no qualitative difference in the response of the immature nervous system, compared to the adult nervous system, with respect to the cytotoxicity of these compounds.

Our results, combined with previous results [7], demonstrate that guanethidine and structurally very similar compounds (2, 3, 5 and 7) produce sympathectomy *in vivo*. This may be due largely to the fact that only compounds very similar to guanethidine accumulate significantly in the cell bodies' sympathetic neurons. Perhaps most striking in this regard is the very high accumulation of compound 3 compared to compound 4, which does not accumulate in SCG. The striking differences in accumulation in the cell bodies do not correlate with adrenergic neuron blocking activity [10] or affinity for the sodium-dependent uptake pump on which blocking activity is dependent. Since the accumulation and cytotoxicity of guanethidine in the ganglia are blocked by desmethylimipramine [6], it would appear that uptake by the sodium-dependent pump is necessary but not sufficient for subsequent accumulation to a high concentration in the cell body. The data in Table 1 suggest that accumulation to a high concentration in the cell body is dependent upon the presence of a ring nitrogen separated from the guanidinium group by a 2 carbon bridge. Increasing the carbon bridge to 3 carbons reduced accumulation by 80 per cent (compare compound 3 and compound 12).

Since the majority of compounds do not accumulate in sympathetic neuron perikarya and hence cannot exert a cytotoxic effect, they are not useful as tools in elucidating the molecular mechansim involved in cytotoxicity. Two compounds (8 and 12) were found which do accumulate to a significant level but are not cytotoxic. Our results with respect to compound 8 confirm those of Juul [7] in studies using adult animals. These results demonstrate that accumulation is necessary but not sufficient for producing cytotoxicity.

Since inhibition of oxidative phosphorylation has been proposed as a possible mechanism of cytotoxicity, we examined the relative potencies of several compounds with respect to this activity, with particular attention to compound 8 which accumulates but is not cytotoxic. If this were the mechanism of toxicity, compound 8 would be expected to be much weaker than guanethidine as an inhibitor of oxidative phosphorylation. It was found that compound 8, rather than being weaker, was a much more potent (12 ×) inhibitor of oxidative phosphorylation than was guanethidine. This result makes it appear highly unlikely that the demonstrated ability of these compounds to inhibit oxidative phosphorylation in isolated mitochondria can account for the cytotoxic effects produced *in vivo*. This conclusion is also supported by the observation that compounds 2 and 3 are very weak inhibitors of oxidative phosphorylation but are still cytotoxic.

Fields and Pressman [15] concluded that, in a series of alkyl guanidines, the more lipophilic compounds are taken up by mitochondria in the energized state. This results in a low apparent K_i. Some data show that reversal of site I electron transport inhibition occurs as the compound diffuses out after de-energization of the mitochondria by DNP [15]. The I_{50} values in Table 3 were obtained with energized mitochondria and, therefore, should have shown maximal inhibition and been analogous to *in vivo* mitochondria. The slow onset of inhibition is consistent with gradual uptake, but the failure of DNP to gradually release respiration with glutamate is not. Possibly the time period during which we were able to follow the respiration after DNP was too short. Absence of release by DNP is consistent with the proposal [15] that the effect of alkyl guanidines is directly on electron transport at site I rather than on phosphorylation mechanisms.

In summary, guanethidine and close analogs are the only guanidinium adrenergic neuron blocking agents which destroy sympathetic neurons. This may be due to the fact that only these compounds accumulate in the neuronal cell bodies. Accumulation in the cell body appears necessary but not sufficient for cytotoxicity, the most glaring example being Ph881/7 (compound 8). Any proposed mechanism of cytotoxicity must account for the lack of activity of this compound. By measuring accumulation *in vivo*, and comparing with *in vitro* activity on isolated mitochondria, inhibition of oxidative phosphorylation appears not to be the mechanism by which these drugs kill sympathetic neurons. Hence, the mechanism of guanethidine-induced sympathectomy and the mechanism by which nerve growth factors prevent cell death remain unexplained.

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