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EFFECTS OF CHRONIC CHLORISONDAMINE ADMINISTRATION ON THE SYMPATHO-ADRENAL AXIS

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Abstract—The chronic administration of chlorisondamine, a ganglionic blocking agent, for periods up to 1 week produced dose-dependent increases in rat adrenal catecholamines (CA), tyrosine hydroxylase (TH) activity and dopamine β-hydroxylase (DBH) activity, and also increased plasma levels of circulating DBH. Uptake of [^{3}H]epinephrine into isolated adrenal medullary storage vesicles from rats given chlorisondamine was reduced compared to controls. Prior denervation of the adrenal eliminated the chlorisondamine-induced increases in CA and TH, and reduced but did not eliminate the increases in DBH. Atropine pretreatment did not reduce the stimulatory effects of chlorisondamine. Ganglionic blockade was effective throughout the period of chronic chlorisondamine administration, as confirmed by the failure of insulin to evoke CA secretion even when given at the longest time period between chlorisondamine injections. These data suggest that chlorisondamine produces a mixture of direct and trans-synaptic sympatho-adrenal stimulation as well as ganglionic blockade, and that the mixed effect persists upon chronic administration.

Chlorisondamine is a long-acting ganglionic blocking agent which has been used clinically for the treatment of hypertension and in the laboratory as a pharmacological tool. Administration of chlorisondamine to rats results in physiological action consistent with blockade of autonomic transmission [1, 2]; at the level of sympathetic neurotransmitter regulation, chlorisondamine has been shown to lower serum catecholamine levels [3] and to block the adrenal catecholamine-secreting effect of reflex sympathetic stimulators [4–6]. Despite the clear-cut capability of chlorisondamine to interrupt ganglionic transmission, a number of findings have been reported which are inconsistent with simple blockade. While chlorisondamine (and other ganglionic blockers) indeed prevents reserpine-induced acute secretion of adrenal catecholamines, the blockers themselves cause tyrosine hydroxylase (TH) induction [4]. Similarly, adrenal dopamine levels (an indicator of stimulation of catecholamine synthesis) increase within 1 hr of chlorisondamine administration [7], and plasma dopamine β-hydroxylase (DBH), a putative indicator of sympathetic tone, does not decline after six injections of chlorisondamine administered over a 48-hr period, despite marked reductions in plasma catecholamines [3]. These data all suggest that chlorisondamine may produce a mixture of stimulation and blockade.

The current study was undertaken to demonstrate on the biochemical level whether chlorisondamine actually does exhibit a stimulatory action, at what point stimulation may occur, and whether stimulation persists upon chronic administration.

MATERIALS AND METHODS

Treatment of animals. Male Sprague–Dawley rats weighing 200–250 g were given subcutaneous injections of chlorisondamine chloride dissolved in 0.25 ml saline, while controls received saline alone. Doses ranged from 2.5 to 20 mg/kg, given twice daily for periods up to 1 week. In some experiments, rats were pretreated with atropine sulfate (10 mg/kg s.c.) 30 min before each chlorisondamine injection, while in other experiments, the left adrenal was denervated 10 days prior to commencement of chlorisondamine administration. To test the effectiveness of ganglionic blockade, rats receiving saline or chlorisondamine (20 mg/kg) for 3 days were starved overnight and insulin (5 I.U./kg i.v.) was administered 10 hr after the last chlorisondamine injection; animals were killed 2 hr later.

Treatment of tissues. Rats were killed by decapitation after 1, 2, 3 or 7 days of chlorisondamine treatment (in each case 12 hr after the last injection) and blood was collected for determination of plasma DBH activity. Adrenals from each animal were homogenized in 2.5 ml of 0.3 M sucrose containing 25 mM Tris (pH 7.4) and 10 μM iproniazid. Homogenate (0.1 ml) was added to 1.9 ml of 3.5% perchloric acid (PCA), centrifuged at 26,000 *g* for 10 min and the supernatant analyzed for catecholamines. Homogenate (0.5 ml) was added to an equal volume of water containing 2000 units/ml of beef catalase (Sigma) and used for DBH assays. The remainder of the homogenate was centrifuged at 800 *g* for 10 min, and 1 ml of the supernatant was used for determination of [^{3}H]epinephrine uptake (*vide infra*). The rest of the 800 *g* supernatant was centrifuged at 26,000 *g* for 10 min to sediment the catecholamine storage vesicles and the supernatant used for duplicate determinations of TH activity. This fractionation procedure has been described in detail previously [8].

The abilities of the storage vesicles in the 800 *g* supernatant to incorporate [^{3}H]epinephrine were determined by standard techniques [9]. For each adrenal preparation, duplicate tubes were prepared

containing 0.5 ml of the 800 *g* supernatant, 5 μmoles ATP and Mg^{2+}, 5 μCi epinephrine[7-^{3}H] isotopically diluted with 0.1 μmole of unlabeled epinephrine bitartrate, and sucrose–Tris in a final volume of 1 ml. Samples were incubated for 30 min at 30° while the duplicates were kept on ice to serve as blanks. Uptake was stopped by the addition of 2 ml of ice-cold sucrose–Tris, and labeled vesicles were sedimented at 26,000 *g* for 10 min. The supernatant was deproteinized with an equal volume of 7% PCA, centrifuged and analyzed for catecholamines and for radioactivity by liquid scintillation spectrometry; this enabled determination of the specific activity of the labeling medium. The vesicular pellet was washed with fresh sucrose–Tris and recentrifuged twice and then suspended in 3 ml of 3.5% PCA, centrifuged and the supernatant analyzed for catecholamines and radioactivity. Although contaminating particles are present, under these conditions labeling occurs solely in the storage vesicles [10]. The temperature-dependent uptake was calculated as described previously [10] and expressed as uptake per gland (a composite measure reflecting the number of functional vesicles per gland as well as the uptake capability of each vesicle) or as uptake per unit of catecholamines (a measure of the abilities of individual vesicles to incorporate [^{3}H]epinephrine relative to endogenous content, independently of the number of vesicles present).

For studies of the effects of chlorisondamine *in vitro*, vesicles and enzymes from control animals were incubated with and without 1 mM chlorisondamine, and uptakes and activities determined as already described.

Assays. Catecholamines were determined by the trihydroxyindole method using an autoanaylzer, and radioactivity was measured by liquid scintillation spectrometry [10, 11]. Rat adrenal tyrosine hydroxylase activity was determined by the method of Waymire *et al.* [12] using 100 μM tyrosine[1-^{14}C], with activity expressed as nmoles $^{14}CO_2$ evolved per hr; dopa decarboxylase activity was measured by the method of Waymire *et al.* [12] using hog kidney as the enzyme source and 33 μM dopa[1-^{14}C] as substrate, with activity expressed as nmoles $^{14}CO_2$ evolved per hr; monoamine oxidase (water homogenates of rat adrenal without iproniazid) was assayed according to Laduron and Belpaire [13] using 10 μM tyramine[G-^{3}H] as substrate, with activity expressed as nmoles [^{3}H]*p*-hydroxybenzaldehyde formed per hr; dopamine β-hydroxylase activity was measured by a modification [14] of the method of Friedman and Kaufman [15], using 10 μM tyramine[G-^{3}H] as substrate and either, 0.5 mM *p*-hydroxymercuribenzoate (adrenal) or 1.5 mM *N*-ethylmaleimide + 4 μM $CuSO_4$ (plasma) to inactivate endogenous inhibitors, with activity expressed as nmoles [^{3}H]octopamine formed per hr.

Materials. Rats were obtained from Zivic–Miller Laboratories. Chlorisondamine chloride was provided by Ciba Pharmaceuticals. Atropine sulfate, tyramine hydrochloride, *p*-hydroxymercuribenzoate and *N*-ethylmaleimide were obtained from Sigma Chemical Corp., regular insulin (80 I.U./ml) was obtained from Squibb Pharmaceuticals and epinephrine bitartrate from Winthrop Laboratories. Epinephrine[7-^{3}H], tyramine[G-^{3}H], dopa[1-^{14}C] and tyrosine[1-^{14}C] were purchased from New England Nuclear Corp.

RESULTS

Chronic administration of chlorisondamine led to dose-dependent increases in adrenal catecholamines (CA), tyrosine hydroxylase activity (TH) and dopamine β-hydroxylase activity (DBH) (Fig. 1). The magnitudes and time courses of the increases differed for each parameter; the CA and DBH increases required high doses and several days of treatment, while TH was elevated after 3–7 days at low doses and after only 1 day at 20 mg/kg. Chlorisondamine (20 mg/kg) had little or no effect on vesicular uptake of [^{3}H]epinephrine after 1 day of treatment, but with chronic administration there was a marked decrement both in uptake per gland and in uptake per unit of CA (Table 1).

To determine the role of neural input in the chlorisondamine-induced changes in adrenal CA, TH and DBH, rats with one adrenal denervated were given 20 mg/kg for 3 days (Table 2). Prior denervation completely blocked the elevations in CA and TH and reduced the increase in DBH, while in the same animals CA, TH and DBH levels were all increased in the innervated gland.

The possibility that muscarinic receptor stimulation could be involved in the stimulatory effects of chlorisondamine was tested by pretreatment of the rats with

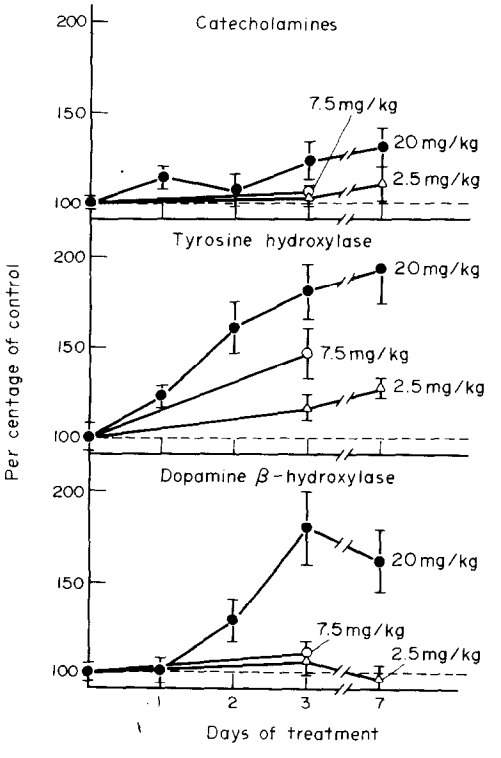

Fig. 1. Effect of chronic treatment with chlorisondamine (twice daily, subcutaneously at indicated doses) on the rat adrenal medulla. Points and vertical bars represent means ± S.E.M. of between 5 and 30 determinations. Control values were: catecholamines, 12.4 ± 0.5 μg/gland; tyrosine hydroxylase, 11.1 ± 0.9 nmoles/gland/hr; dopamine β-hydroxylase, 0.93 ± 0.05 nmoles/gland/hr.

Table 1. Effect of chlorisondamine (20 mg/kg s.c. twice daily) on [^{3}H]epinephrine uptake into isolated adrenal medullary vesicles

Days of treatment	Epinephrine uptake (nmoles/gland)	Epinephrine uptake (nmoles/100 μg endogenous catecholamines)	No.. of determinations
Control	2.38 ± 0.16	21.1 ± 1.5	12
	Control (%)		
0	100 ± 7	100 ± 7	12
1	112 ± 12	85 ± 3	5
7	76 ± 7*	58 ± 4†	10

* $P < 0.01$ vs control. † $P < 0.001$ vs control.

large doses of atropine (Table 3). Atropine alone had no effect on CA, TH or DBH and did not interfere with the chlorisondamine-induced increases. To determine whether animals receiving 20 mg/kg twice daily maintained ganglionic blockade throughout chronic treatment, rats were given insulin 10 hr after the last chlorisondamine injection and sacrificed 2 hr later; insulin-induced hypoglycemia evokes massive sympatho-adrenal stimulation [5]. Both control and chlorisondamine-treated rats evidenced hypoglycemic shock after insulin, as exhibited by convulsions. In rats which did not receive chlorisondamine, insulin caused a 60 per cent depletion of adrenal CA (Table 4); chronic chlorisondamine pretreatment completely prevented the depletion, indicating effective ganglionic blockade even at the maximum time period between injections.

Plasma DBH activity demonstrated a similar dose- and time-dependent increase during chronic chlorisondamine administration (Table 5). There was no change in activity after 3 days at 20 mg/kg. However, after 1 week at 2.5 mg/kg, there was a 30 per cent increase in plasma DBH and after 1 week at 20 mg/kg activity tripled.

Chlorisondamine *in vitro* had little or no effect on activities of catecholamine biosynthetic or degradative enzymes, and caused a small degree of inhibition of [^{3}H]epinephrine uptake into isolated storage vesicles (Table 6). Since the concentrations utilized (1 mM) are considerably higher than that which should be achieved with the highest dose *in vivo*, it is unlikely that direct actions of chlorisondamine on enzymes and vesicles are responsible for the results obtained after chronic administration to rats.

Table 4. Effect of chlorisondamine (20 mg/kg s.c. twice daily for 3 days) on insulin-induced secretion from the rat adrenal medulla*

Treatment	Catecholamines (μg/gland)	No. of determinations
Control	10.4 ± 1.0	6
Insulin	4.18 ± 0.40†	5
Chlorisondamine + insulin	11.8 ± 0.7	4

* Insulin (5 I.U./kg i.v.) was given 10 hr after the last chlorisondamine injection and rats were sacrificed 2 hr later.

† $P < 0.001$ vs control.

Table 2. Influence of prior denervation on effects of chlorisondamine on the rat adrenal medulla

Treatment	Catecholamines (μg/gland)		Tyrosine hydroxylase (nmoles/gland/hr)		Dopamine β-hydroxylase (nmoles/gland/hr)		No. of determinations
	Innervated	Denervated	Innervated	Denervated	Innervated	Denervated	
Control	10.0 ± 0.5	9.86 ± 0.37	16.3 ± 1.3	16.4 ± 1.4	0.858 ± 0.056	0.807 ± 0.035	6
Chlorisondamine (20 mg/kg s.c., twice daily for 3 days)	12.4 ± 0.6*	10.5 ± 0.3	35.0 ± 2.4†	13.9 ± 1.2	1.47 ± 0.14‡	1.08 ± 0.11§	5

* $P < 0.02$ vs control. † $P < 0.001$. ‡ $P < 0.005$. § $P < 0.05$.

Table 3. Influence of atropine pretreatment on the effects of chlorisondamine on the rat adrenal medulla*

Treatment	Catecholamines (μg/gland)	Tyrosine hydroxylase (nmoles/gland/hr)	Dopamine β-hydroxylase (nmoles/gland/hr)	No. of determinations
Control	9.91 ± 0.47	8.55 ± 0.72	0.821 ± 0.065	5
Atropine alone	9.59 ± 0.60	7.78 ± 0.54	0.662 ± 0.019	5
Chlorisondamine alone	10.6 ± 0.9	13.8 ± 1.2†	1.06 ± 0.10	5
Atropine + chlorisondamine	10.3 ± 0.6	16.5 ± 2.2‡	1.13 ± 0.08§	4

* Rats were given twice daily subcutaneous injections of chlorisondamine (20 mg/kg) or atropine (10 mg/kg) for 2 days. With combined treatment, atropine was given 30 min prior to chlorisondamine.

† $P < 0.01$ vs control.

‡ $P < 0.02$ vs control; not significant vs chlorisondamine alone.

§ $P < 0.05$ vs control; not significant vs chlorisondamine alone.

Table 5. Effects of chlorisondamine (s.c. twice daily) on rat plasma dopamine β-hydroxylase

Treatment	Plasma DBH (nmoles/hr/l.)	No. of determinations
Control	22.2 ± 1.4	12
2.5 mg/kg, 7 days	28.6 ± 1.3*	6
20 mg/kg, 3 days	22.3 ± 0.8	6
20 mg/kg, 7 days	69 ± 21†	6

* $P < 0.005$ vs control.
† $P < 0.01$.

Table 6. Effects of chlorisondamine (1 mM) *in vitro* on catecholamine biosynthetic and degradative enzymes and on [^{3}H]epinephrine uptake into adrenal medullary vesicles*

Determination	Inhibition (%)
Tyrosine hydroxylase†	16 ± 7
Dopa decarboxylase‡	0 ± 3
Dopamine β-hydroxylase§	1 ± 3
Monoamine oxidase‖	1 ± 3
[^{3}H]epinephrine uptake¶	25 ± 5**

* Four determinations were made for each point.
† Rat adrenal; control activity 15.3 ± 0.5 nmoles/hr/ml of preparation.
‡ Hog kidney; control activity 201 ± 16 nmoles/hr/ml of preparation.
§ Rat adrenal; control activity 0.681 ± 0.20 nmole/hr/ml of preparation.
‖ Rat adrenal; control activity 2.31 ± 0.04 nmoles/hr/ml of preparation.
¶ Rat adrenal vesicles; control uptake 12.0 ± 0.3 nmoles/100 μg of endogenous catecholamines.
** $P < 0.005$.

DISCUSSION

Stimulation of the sympatho-adrenal axis is accompanied by changes in the biochemistry of CA synthesis, uptake, storage and release. Thus, after the acute administration of reserpine, insulin or nicotine, there is induction of the CA biosynthetic enzymes, TH and DBH, and an increase in the synthesis of new CA storage vesicles [4–6, 8–10, 16]. Adrenal CA levels may be decreased, unchanged or even increased, depending upon the relative balance between stimulation-induced CA release and stimulation-induced increases in CA synthesis and storage. For example, acute administration of large doses of nicotine produces adrenal CA depletion, but upon chronic administration CA levels increase [16]; other doses of nicotine can be chosen which produce little change in CA levels but which still cause TH and DBH induction [16].

In the present study, results have been obtained which indicate that chronic administration of chlorisondamine, a ganglionic blocking agent, produces also a stimulatory effect in the sympatho-adrenal axis. This is demonstrated by the dose-dependent increases in adrenal TH and DBH activity and in CA levels, a biochemical pattern which is identical in every respect to that after chronic administration of nicotine [16]. These results cannot be accounted for by a possible muscarinic stimulation by chlorisondamine, since atropine pretreatment did not prevent the chlorisondamine-induced biochemical changes.

Chronic chlorisondamine treatment also produced changes in the functioning of CA storage vesicles, as evidenced by the reduction in vesicular uptake. The uptake decrease probably results from TH induction, with a consequent increase in the CA content of each vesicle and thus a decline in uptake/100 μg of CA; it should be noted that the higher level of uptake per gland vs uptake/100 μg of CA ($P < 0.05$) provides corroborative evidence that the number of vesicles is increased (as suggested by DBH induction) while uptake per vesicle is decreased. A similar pattern of changes in uptake has been observed after chronic nicotine or morphine administration [16, 17], both of which, like chlorisondamine, have little or no effect on uptake *in vitro*. In contrast, outright inhibition of uptake by reserpine results in a greater or equal decrease in uptake per gland and per unit of CA [6].

To evaluate further the sympatho-adrenal stimulation by chlorisondamine, plasma DBH activity was determined; plasma DBH is presumed to come from sympathetic nerves and to a lesser extent from the adrenal medulla [18] and thus has been suggested as an index of sympatho-adrenal activity. Chronic administration of chlorisondamine produced a dose-dependent increase in plasma DBH, and the increase required a somewhat longer period of treatment than for adrenal changes. These data are consistent with sympathetic stimulation and further suggest that the time courses of the biochemical changes may vary with the tissue studied.

It was important to determine whether chlorisondamine stimulates sympathetic tissues directly (like nicotine) or whether the biochemical changes are trans-synaptic. Mueller *et al.* [4] have reported that the increase in adrenal TH caused by acute chlorisondamine administration could be prevented by prior sectioning of the splanchnic nerve, thus implicating a purely trans-synaptic mechanism. In the current studies, we have obtained evidence after chronic administration which partially corroborates this hypothesis. Prior denervation completely prevented the chronic chlorisondamine-induced increases in TH and CA, and reduced, but did not eliminate, the induction of DBH. Similar results have been obtained with denervated adrenals after morphine or reserpine administration [19, 20]. These data indicate that the stimulation during chronic chlorisondamine administration has both trans-synaptic and direct components, and the levels of DBH (in contrast to TH) may be regulated in part by a non-trans-synaptic mechanism. The trans-synaptic component may result from reflex compensatory mechanisms, as suggested by Maxwell *et al.* [2], or from direct presynaptic release of acetylcholine, as demonstrated for another ganglionic blocking agent, tetraethylammonium [21].

There are two time points at which stimulation is likely to occur: if the response is due directly to chlorisondamine, stimulation should occur early (when drug concentrations are high); if the response is due to rebound stimulation from a wearing off of ganglionic blockade, stimulation should occur late, when drug concentrations might be low enough to permit ganglionic transmission. The latter hypothesis can be

ruled out, since insulin administration, even at the time when chlorisondamine concentrations would be at their lowest (10–12 hr after the last dose), failed to evoke CA release; this indicates that ganglionic blockade did not wear off between doses. The contention that stimulation occurs early after chlorisondamine administration is supported by the observation of Carlsson and Lindqvist [7] that adrenal dopamine levels (an indicator of short-term, stimulation-induced TH activation) show a peak 60–90 min after chlorisondamine; this corresponds to the time period in which blood pressure returns to normal in conscious rats given chlorisondamine [2].

In conclusion, it is evident that the effects of chlorisondamine constitute a mixture of direct and transsynaptic sympatho-adrenal stimulation as well as ganglionic blockade. Because of the relatively long half-lives of CA biosynthetic enzymes and storage vesicles (hours to days [6, 9, 10, 22]), the short bursts of stimulation which accompany each dose may be sufficient to maintain elevated tissue levels of CA, TH, DBH and vesicles. Consequently, the chronic use of chlorisondamine to interrupt sympathetic transmission can yield results which are difficult to interpret on the basis of ganglionic blockade alone. It is of interest that Pardo and Vidrio [23] have demonstrated that chronic administration of chlorisondamine or mecamylamine to dogs results eventually in sustained elevations in blood pressure; thus, sympatho-adrenal stimulation accompanying long-term treatment with chlorisondamine may be of functional significance.

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