Atropine Antagonism of Carbachol-Induced Hyperglycemia

By A. E. WADE

Hyperglycemia was induced with carbachol in rats, rabbits, and dogs, and the effect in rabbits was shown to be dose related. In albino rabbits with negligible plasma esterase activity, atropine sulfate effectively prevented hyperglycemia following 25 mcg./Kg. of carbachol, and the effect appeared to be dose dependent. In rabbits with moderate esterase levels, atropine sulfate dosage as high as 0.8 mg./Kg. had little inhibitory effect on carbachol hyperglycemic or cholinomimetic responses. This plasma esterase was not effectively blocked *in vivo* with 20 mg./Kg. of 2-(diethylamino)ethyl-2,2-diphenylvalerate (proadifen) HCl (SKF 525-A). However, this dose of proadifen HCl did block carbachol hyperglycemia in rabbits with negligible esterase activity. Carbachol hyperglycemia was similarly prevented by atropine in rats and dogs.

HEPATIC PHOSPHORYLASE ACTIVITY glycogenolysis, and the hyperglycemia resulting from the administration of exogenous epinephrine are reduced by certain adrenergic blocking agents (1–8) and certain indirectly acting sympathomimetics (2). The biochemical site of action of these dissimilar agents appears to be different (9), some suppressing synthesis of adenosine-3′,5′-monophosphate and others affecting reactions subsequent to this.

Catecholamines may be liberated from the adrenal medulla as a result of hypoglycemia (10) or by drugs such as acetylcholine, carbachol, histamine, nicotine, physostigmine, or reserpine (11, 12). Suppression of catecholamine mobilization from adrenal stores by these stimuli has been accomplished with chronic administration of reserpine and monoamine oxidase inhibitors, by ganglionic blocking agents, and in certain instances by atropine, chlorpromazine, guanethidine, and tetracaine (12).

Atropine has been shown to reduce blood levels of catecholamines (13), block the pressor response of neostigmine (14–16) and other ganglionic stimulants (16, 17), and depress the response of the nictitating membrane to nerve stimulation (18). The site of action is presumably at the ganglion. Atropine in large doses has adrenolytic activity, reducing many effects of exogenous epinephrine and sympathetic nerve stimulation (19, 20).

The effects of atropine on carbachol hyperglycemia in rabbits, rats, and dogs are enumerated in this report.

EXPERIMENTAL

The animals used in this study included female albino rabbits of the California strain, weighing 1.2 to 5.3 Kg.; female Sprague-Dawley rats, weighing 170–251 Gm.; and mongrel dogs of both sexes, weighing 8–14.5 Kg.

Serum esterase levels of the rabbits were determined by the method outlined previously (21). The serum esterase level was considered negligible if, under the conditions of this assay, less than 0.06 μ-mole procaine was hydrolyzed per ml. serum per hour at 26 ± 1°. Following an 18–24-hr. fast, rabbits were anesthetized with i.p. injections of a solution of Na phenobarbital (100 mg./Kg.) and Na pentobarbital (15 mg./Kg.) dissolved in 50% propylene glycol. Approximately 1 hr. after anesthesia was established, 0.9% NaCl solution, atropine sulfate, or 2-(diethylaminoethyl)-2,2-diphenylvalerate (proadifen) HCl (SKF 525-A)[1] was administered i.v. Blood samples of 0.6 to 1.0 ml. were removed by cardiac puncture 10 min. after such pretreatment and 10 min. prior to the intravenous carbachol.[2] Additional blood samples were obtained at 15, 30, and 60 min. following carbachol. The samples were mixed with dry sodium citrate and immediately placed in an ice–salt bath until deproteinized.

Rats were fasted overnight and anesthetized with i.p. injections of 25 mg./Kg. Na pentobarbital and 75 mg./Kg. Na phenobarbital in 50% propylene glycol. Blood samples (0.5 ml.) were removed by cardiac puncture 20 min. prior to the subcutaneous administration of 0.9% NaCl or atropine $SO_4^=$ and 35 min. following the subcutaneous administration of 25 mcg. of carbachol per Kg. body weight.

Dogs were fed 18–20 hr. prior to i.p. anesthesia with a solution of Na pentobarbital (10 mg./Kg.) and Na phenobarbital (120 mg./Kg.) in 50% propylene glycol, and 3 hr. later atropine $SO_4^=$ (0.4 mg./Kg.) was administered subcutaneously to the experimental group. Blood samples were withdrawn from the cephalic vein 20 min. prior to subcutaneous carbachol (25 mcg./Kg.) administration, and at 10, 20, and 50 min. after carbachol. In some experiments epinephrine HCl (10 mcg./Kg.) was administered 120 min. following carbachol in order to validate the animals' ability to mobilize hepatic glycogen.

Glucose content of 0.1-ml. samples of deproteinized blood was determined in duplicate by the method of Washko and Rice (22) using a prepared enzyme reagent.[3]

Drug effects were based on the quantitative change in mg. per cent of glucose from pre-carbachol injection values. Each point on the dose-response curves representing total measured response to a given treatment was derived by calculating the area under the graph produced by plotting change in glucose content (mg. %) during the 60 min. post-carbachol. One factor analysis of variance was conducted on all data using standard electronic data processing equipment by the University of Georgia Bureau of Statistics. Differences between groups were calculated at the 5% level using the Duncan multiple range test.

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[1] SKF 525-A was graciously supplied by Smith, Kline & French Laboratories, Philadelphia, Pa.

[2] Marketed as Carcholin by Merck & Co., Rahway, N. J.

[3] Marketed as Glucostat by Worthington Biochemical Corp., Freehold, N. J.

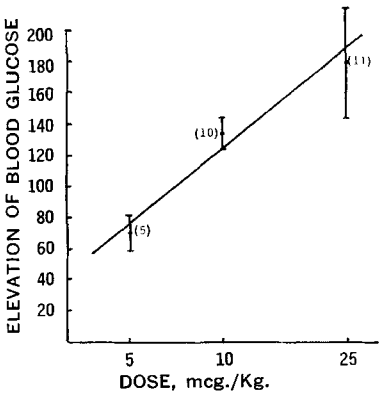

Fig. 1—Total blood glucose elevation during 60-min. post-carbachol in rabbits. Values and bars indicate mean ± S.E. Numbers in parentheses indicate number of animals per dose.

RESULTS

Carbachol Hyperglycemia—The log dose-response curve for carbachol-induced hyperglycemia over the 60-min. period following i.v. carbachol administration in rabbits is il ustrated in Fig. 1. The response is clearly dose dependent, and although 25 mcg./Kg. of carbachol appeared to be supramaximal in some animals, this dose produced hyperglycemia significantly greater than that resulting from 5 mcg./Kg. The hyperglycemia was significant for all doses of carbachol at 15 and 30 min. post-injection, but at the 60-min. period the blood glucose concentration had returned to a level not significantly higher than the controls administered 0.9% NaCl solution. All doses produced profound parasympathomimetic actions, but seldom produced death if infused over a 60–90-sec. period.

Atropine Inhibition of Carbachol Hyperglycemia—The inhibitory effect of atropine sulfate on the hyperglycemia induced by carbachol in rabbits exhibiting negligible serum esterase activity is recorded in Table I. This effect of atropine appeared to be dose related, since 0.05 mg./Kg. produced 70.8% inhibition of the carbachol hyperglycemia and 0.10 mg./Kg. resulted in a 92% inhibition. Doses of 0.2 and 0.4 mg./Kg. produced inhibition statistically indistinguishable from that produced by 0.1 mg./Kg. All doses of atropine sulfate above 0.05 mg./Kg. maintained blood glucose levels in the presence of carbachol that were not statistically different from the nontreated (saline injected) controls.

The mean hyperglycemic response to carbachol was less pronounced in atropinized rabbits having innately high serum esterase levels than in nonatropinized controls; however, this effect was not significant (Fig. 2). Data are also presented in Fig. 2 which suggest that proadifen HCl produces an effect resembling that of atropine but that it does not effectively block the esterase responsible for atropine hydrolysis. This ineffectiveness as a blocking agent was explained, at least in part, by *in vitro* experiments which demonstrated that proadifen HCl is inactivated by rabbit serums having atropinesterase activity. *In vivo* studies in rabbits with negligible esterase levels demonstrated the atropine-like effect of proadifen HCl on carbachol-induced hyperglycemia (Fig. 3).

In the rat, atropine sulfate (0.05 to 0.4 mg./Kg.) produced complete blockade of the hyperglycemia of carbachol. This blockade, measured at 50–55 min. following subcutaneously administered atropine, was not evident at 80 min. post-atropine.

The effect of atropine sulfate in the dog is depicted in Fig. 4. Complete blockade of carbachol hyperglycemia with this dose of atropine sulfate seems evident, although statistically significant changes in blood glucose concentrations were not measured until 50 min. post-carbachol. It became evident early in this study with dogs that glycogen reserves are quickly depleted and that heavy feeding 14–16 hr. prior to the experiments supplied insufficient glycogen reserves to allow consistent hyperglycemia following carbachol injections. Priming doses of orally administered dextrose (3 Gm./Kg.) approximately 3 hr. prior to the experiments alleviated this difficulty and provided sufficient hepatic glycogen to allow measurable hyperglycemia in response to epinephrine in both atropinized and control animals.

DISCUSSION

Carbachol induces hyperglycemia in rabbits, rats, and dogs which is probably due to the liberation of epinephrine from adrenal medullary stores. The data reported here indicate that the mechanism by which atropine inhibits this hyperglycemia is similar in these animals. Although the site of action was not specifically located, it seems reasonable to assume that atropine blocks at a point preceding the release of epinephrine, since it was shown that atropine did not prevent the hyperglycemia caused by a dose of 10 mcg./Kg. of epinephrine in dogs that had failed to respond to carbachol.

TABLE I—EFFECT OF ATROPINE $SO_4^=$ ON CARBACHOL (25 mcg./Kg. i.v.) HYPERGLYCEMIA IN RABBITS WITH NEGLIGIBLE SERUM ESTERASE ACTIVITY

No. of Animals in Group	Pretreatment	Change in Blood Glucose (mg. %) from Pre-Carbachol Levels— 15 min.	30 min.	60 min.
11	Saline	59.43 ± 9.70	55.89 ± 13.58	36.68 ± 8.88
8	Atropine $SO_4^=$ 0.05 mg./Kg.	12.46 ± 5.51[a]	12.31 ± 4.66[a]	21.89 ± 8.98
6	Atropine $SO_4^=$ 0.10 mg./Kg.	6.25 ± 7.18[a]	4.18 ± 5.48[a]	1.90 ± 4.89[a]
5	Atropine $SO_4^=$ 0.20 mg./Kg.	3.28 ± 4.80[a]	2.36 ± 3.44[a]	3.74 ± 4.73[a]
6	Atropine $SO_4^=$ 0.40 mg./Kg.	4.05 ± 1.86[a]	2.57 ± 3.95[a]	1.35 ± 3.80[a]
7	No carbachol-saline	−2.21 ± 5.13[a]	−2.17 ± 3.60[a]	1.09 ± 9.05[a]

[a] Significantly different from carbachol controls.

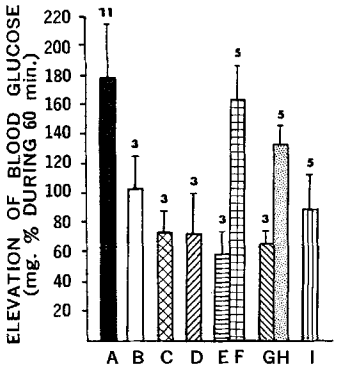

Fig. 2—Effect of atropine SO_4^- and atropine SO_4^- with proadifen HCl (20 mg./Kg.) on the hyperglycemia of carbachol (25 mcg./Kg.) in rabbits with innately high serum esterase levels. Vertical bars represent standard errors of the mean. The number above each bar indicates number of animals per group. Key: A, *control;* B, *proadifen HCl;* C, *proadifen HCl + atropine SO_4^- (0.05 mg./Kg.);* D, *proadifen HCl + atropine SO_4^- (0.10 mg./Kg.);* E, *proadifen HCl + atropine SO_4^- (0.20 mg./Kg.);* F, *atropine SO_4^- (0.20 mg./Kg.);* G, *proadifen HCl + atropine SO_4^- (0.40 mg./Kg.);* H, *atropine SO_4^- (0.40 mg./Kg.);* I, *atropine SO_4^- (0.80 mg./Kg.).*

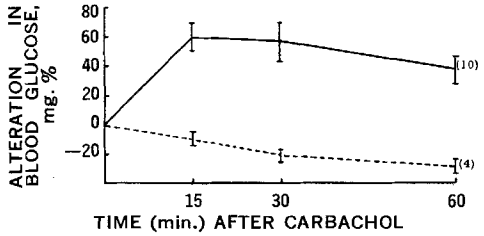

Fig. 3.—The effect of proadifen HCl (20 mg./Kg. i. v.) on carbachol (10 mcg./Kg.) hyperglycemia in rabbits with negligible serum esterase. Numbers in parentheses indicate number of animals per group. Key: ————, *control;* – – – – – –, *proadifen HCl pretreated.*

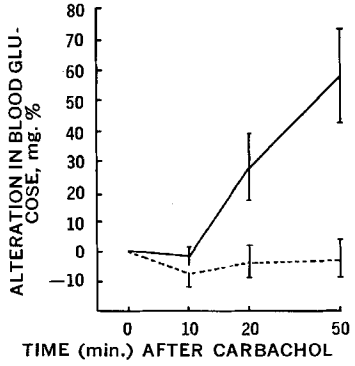

Fig. 4—The effect of atropine SO_4^- (0.4 mg./Kg. s. c.) on carbachol (25 mcg./Kg. s. c.) in the dog. The values and bars represent mean ± S. E. of 4 experiments. Key: ————, *control;* – – – – – –, *atropine pretreatment.*

The enzyme inhibitor proadifen HCl has been reported to inhibit human serum procainesterase activity (23). This inhibition was verified during an *in vitro* study which demonstrated that 1×10^{-4} *M* proadifen HCl reduced procaine hydrolysis by human serum by about 61%. *In vitro* experiments indicated, however, that proadifen HCl at concentrations of 1.4×10^{-3} *M* does not inhibit hydrolysis of atropine by esterase of rabbit serum, and in fact serves as a substrate for this enzyme. The fact that proadifen HCl does not effectively block atropinesterase activity was verified in the *in vivo* experiments in rabbits with high serum esterase levels. The anticholinergic activity of proadifen HCl is well known and its atropine-like activity in blocking carbachol hyperglycemia was demonstrated in these studies. Thus, the antihyperglycemic activity of atropine and proadifen HCl in rabbits with high esterase activity appears to be no more than an additive effect of the two drugs.

If the antihyperglycemic action of atropine is present in man, and if it is capable of inhibiting epinephrine release from the adrenal medulla by stimuli other than carbachol (*i.e.*, hypoglycemia), then two distinct possibilities of clinical importance appear likely. Cholinergic blocking drugs may find use (*a*) in controlling the hyperglycemia of the select group of patients having normal insulin levels but transiently high concentrations of circulating catecholamines, the release of which is triggered by an oversensitive sympathetic hyperglycemic reaction, and (*b*) as insulin-sparing agents for diabetics receiving oral hypoglycemics, by reducing the antagonistic effect of the adrenal medulla. This second point may be doubly important in view of the fact that certain of the oral hypoglycemic agents stimulate epinephrine release from the adrenal medulla (24).

SUMMARY

Carbachol in doses of 5, 10, and 25 mcg./Kg. i.v. produced peak elevations in blood glucose concentration in rabbits within 15 min.

This hyperglycemia was effectively blocked by doses of atropine sulfate as low as 0.05 mg./Kg. in both rabbits with negligible serum esterase activity and in rats. Atropine antagonism of carbachol hyperglycemia was also evident in dogs.

The enzyme inhibitor proadifen HCl (20 mg./Kg. i.v.) completely antagonized the hyperglycemia of carbachol (10 mcg./Kg. i.v.) in rabbits with negligible serum esterase activity, but was ineffective in blocking the esterase of rabbit serum responsible for atropine hydrolysis.

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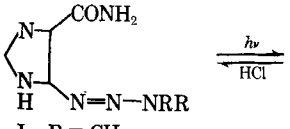
Colorimetric Determination of Dialkyltriazenoimidazoles

By TI LI LOO and ELIZABETH A. STASSWENDER

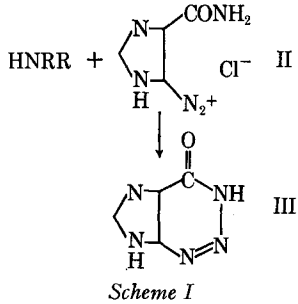
A colorimetric method has been developed for the determination of dialkyltriazenoimidazoles in plasma and urine.

RECENTLY a series of dialkyltriazenoimidazoles (I) has been synthesized as potential antitumor agents (1). Several of these have shown promise as useful drugs in cancer chemotherapy, notably 5-(dimethyltriazeno)-imidazole-4-carboxamide (I*a*) (DIC, NSC-45388) and 5-[di(β-chloroethyl) triazeno]-imidazole-4-carboxamide (I*b*) (NSC-82196) (2,3). The former is presently under clinical trial. Concurrently, pharmacologic studies on these new agents in man and animals are also in progress in our laboratories. For such studies a simple, sensitive, and specific method for the quantitative determination of the dialkyltriazenoimidazoles is desirable. The method described below appears to meet this demand.

The dialkyltriazenoimidazoles undergo photodecomposition in dilute acid to 5-diazoimidazole-4-carboxamide (II) and a corresponding dialkylamine according to Scheme I.



I*a*, R = CH_3–
I*b*, R = $ClCH_2CH_2$–



Scheme I

In aqueous solutions II readily cyclizes by intramolecular coupling to 2-azahypoxanthine (III), imidazo-[4,5-*d*]-*v*-triazin-4(3*H*)-one (1). However, in the presence of an aromatic amine such as *N*-(1-naphthyl)ethylenediamine (Bratton-Marshall reagent) (4), coupling takes place with the formation of an intensely colored azo-dye. Under rigidly controlled experimental conditions with an excess of the coupling amine, the competing intramolecular coupling to III could be held to a constant minimum; thus the intensity of the color adequately measures the concentration of I. Because the photodecomposition is a general reaction of I, the colorimetric method is expected to be applicable to all dialkyltriazenoimidazoles, although the present work concerns mainly I*a* and I*b*.

EXPERIMENTAL

Apparatus

A standard colorimeter (for example, Bausch & Lomb Spectronic 20) is required for measurement of absorbance of 0.4 ml. to 2 ml. of solution.

The light source is a long-wave (maximal radiation at 366 mμ) ultraviolet lamp (Blak-Ray lamp, model XX-15, supplied by Ultraviolet Products, Inc., San Gabriel, Calif., equipped with two General Electric F15 T8-BLB 15-w. bulbs).

A wooden rack holds the test tubes (Pyrex 75 × 10 mm.) containing drug solutions rigidly vertical and at a fixed distance of 2 cm. from the light bulbs. The lamp is positioned so that an entire row of test tubes may receive the same maximal exposure to ultraviolet radiation.

Reagents

Bratton-Marshall Reagent—Aqueous solutions of *N*-(1-naphthyl)ethylenediamine dihydrochloride, 0.2% for aqueous and plasma assays, but 1% for urine assays.

DIC Standards—A stock solution of DIC, 10.0 mg./ml. in 0.1 *N* hydrochloric acid is prepared and stored in the dark under refrigeration. It is stable for at least 1 month. Dilutions are made of stock with 0.1 *N* hydrochloric acid, plasma, or urine.

Trichloroacetic Acid—A 10% solution of trichloroacetic acid in 6 *N* sulfuric acid.

PROCEDURES

Determination in Aqueous Solutions—A mixture of 2.0 ml. of DIC solution, ranging from 0.1 mcg./

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