TABLE VII.—INTERACTION BETWEEN PRESENCE *vs.* ABSENCE OF *N,N'*-DIETHYLTHIOUREA AND PRESENCE *vs.* ABSENCE OF THIOUREA

N,N'-Diethylthiourea	Thiourea	No Thiourea	Differences
Present	196.86	179.83	17.03
Absent	179.93	108.40	71.53
Differences	16.93	71.43	

TABLE VIII.—EFFECTS OF METHODS OF TREATMENTS ON ALKALOID ABSORPTION EXPRESSED IN ABSOLUTE UNITS

Methods	Av.
Food	190.95
Fasting	86.79
Buffer A	212.39
Buffer B	174.99

of *N,N'*-diethylthiourea is greater when thiourea is absent (71.43 units contrasted to 16.93). There is a large statistically significant effect for the interaction $A \times D$. Gross inspection of the lower half of Table II indicates that there is a large effect between *N,N'*-diethylthiourea and quinidine as previously reported (1), and this is the reason for the significant $A \times D$ interaction.

The *Methods of treatments* comparison was statistically significant as indicated in the analysis of variance of Table III. Table VIII indicates that this was almost entirely due to the 24-hour fasting of the rats prior to the test. Fasting definitely hinders absorption of the alkaloids in these experiments. Each figure in Table VIII is the mean of 16 items. The inhibitory effect of fasting can be seen as a uniform phenomenon in Tables V and VI. Inspection of Table VI indicates that 1-phenyl-2-thiourea in a fasted animal encourages alkaloidal absorption as reported earlier (1); yet in a nonfasted animal its presence actually discourages absorption.

SUMMARY

A factorial experiment was carried out in rats to study the effects of thiourea, *N,N'*-diethylthiourea, 1-phenyl-2-thiourea, ionic strength, *ad libitum* food, and 24-hour fasting on the absorption of quinine and quinidine. Thiourea administered in equal weight with quinine consistently gave enhancement of absorption, while absence of effect from thiourea was observed when given with quinidine. 1-Phenyl-2-thiourea administration resulted in lowering the rate of absorption in nonfasted animals while increasing absorption in fasted animals. *N,N'*-Diethylthiourea routinely increased the absorption of both quinine and quinidine. There was no noticeable interference with alkaloidal absorption from either the strongly buffered or the weakly buffered vehicles. Fasting exerted a strong inhibitory effect on quinine and quinidine absorption.

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Influence of Adrenergic Receptors on Blood Sugar and Lactic Acid Levels in the Rat

By BENJAMIN W. LEI* and ROB S. McCUTCHEON

Six treatment combinations were compared for their production of blood sugar and lactic acid in rats. The challenging drugs—saline, epinephrine, and levarterenol-isoproterenol combined—were tested against saline (without adrenergic blockers) and against DCI combined with hydergine. Epinephrine and levarterenol-isoproterenol, the challenging amines, were both effective in increasing the production of blood sugar and lactic acid. The adrenergic blockade produced by DCI combined with hydergine was effective in inhibiting both hyperglycemia and hyperlacticacidemia. Since a specific blockade of both α and β adrenergic receptors prevented the glycogenolytic effects of epinephrine and levarterenol-isoproterenol, it is concluded that this effect is mediated through these receptors.

IT IS WELL known that the sympathetic amines will cause an increased production of both blood sugar and lactic acid (1); there is, however, some uncertainty as to which adrenotropic receptors, if any, are responsible for the mediation of this response. Glycogenolysis was first attributed to the α receptors by Ahlquist (2), then to both receptors by Van der Pol (3) and Claasen and Noach (4). Mayer, *et al.* (5), and McCutcheon (6) took exception to this and suggested that the β receptors—or possibly some unknown receptors

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—were responsible for this glycogenolytic effect. However, Furchgott (7) contends that this response cannot be assigned to either the α or β receptors, and he has suggested a separate class—γ receptors—for glycogenolysis.

Despite this controversy, some observations regarding glycogenolysis have been consistently reported. First, epinephrine, a potent α and β stimulant, produced the greatest increase in blood sugar and lactic acid, whereas isoproterenol, a specific β stimulant, produced the smallest effect. The potency ratio in this regard for the three classical amines are: *l*-epinephrine > levarterenol > *dl*-isoproterenol (8, 9) (Claasen and Noach(4)). Second, no complete blockade of catecholamine-induced glycogenolysis has been demonstrated when using either α or β blocking agents independently. The reported inhibition of this response has varied from relatively ineffective to relatively effective. Third, of all the adrenergic blocking agents used to inhibit this response, the derivatives of the ergot alkaloids have been shown to be the most effective. This class of drugs is considered primarily blockers of the α receptors with some blocking action on the β receptors (10). Fourth, the rise in blood sugar produced by the conjoint administration of levareterenol and isoproterenol, independent α and β stimulants, was greater than that of either alone at the same dosage level (3).

These observations are best explained if it is assumed that glycogenolysis was mediated through the synergistic effects of both α and β receptors. Thus, further evidence for such a hypothesis would be advanced if data could be presented to demonstrate that the combined blockade of the α and β adrenotropic receptors completely inhibited this response.

METHODS

l-Epinephrine bitartrate, levarterenol bitartrate, and *dl*-isoproterenol HCl (Sterling-Winthrop Research Institute) were made up in 0.001 *M* solutions in 0.1% sodium bisulfite and 0.1% chlorobutanol as preservatives. Dichloroisoproterenol, *dl*-1-(3′,4′-dichlorophenyl)-2-isopropylaminoethanol HCl (DCI, Eli Lilly and Co.) was preserved in the same manner. Hydergine (Sandoz, Inc.) was used from ampuls containing 0.1 mg. each of the dihydrogenated ergot alkaloids in 1 ml.

Rats, fasted for 16–18 hours, were anesthetized intraperitoneally with pentobarbital (Abbott Laboratories), 35 mg./Kg. Three rats received 0.1 ml. of saline, and three other rats received the conjoint administration of DCI (10 mg./Kg.) and hydergine (0.2 mg./Kg.). Thirty minutes after pretreatment of the three rats with saline, they were given, (I) saline 0.1 ml., (II) epinephrine (0.2 mg./Kg.) and isoproterenol (0.2 mg./Kg.). The three rats pretreated with the combined α and β adrenergic blockers were treated in exactly the same manner: (IV) saline 0.1 ml., (V) epinephrine (0.2 mg./Kg.), and (VI) levarterenol (0.2 mg./Kg.) conjointly with isoproterenol (0.2 mg./Kg.). Since all doses were given intraperitoneally, the doses used by Van der Pol (3) and Claasen and Noach (4) were reduced to give approximately the same activity according to Ellis (8). The chosen dose of hydergine (0.2 mg./Kg.) was successful in antagonizing the hyperglycemic effect without gross signs of side effects (11).

Chemical Methods.—One hour after treatment, blood collected by cardiac puncture with a 1-ml. tuberculin (precision) syringe coated with heparin (5, 12) was ejected directly into the deproteinizing solution (13). After deproteinization by the method of Van Slyke and Hawkins (14), blood sugar was determined according to the method of Nelson (15), in which an alkaline copper solution is reduced by glucose, an arsenomolybdate color reagent added, and the resulting degree of change determined in a colorimeter. The lactic acid was determined by the method of Barker and Summerson (16), in which glucose and other interfering substances are removed from the protein free filtrate with copper sulfate and calcium hydroxide. The lactic acid is converted to acetaldehyde with sulfuric acid and determined colorimetrically after reaction with *p*-hydroxydiphenyl in the presence of copper ions.

Statistical Method.—This experiment utilized a 2 × 3 factorial design in randomized complete blocks. The combined α and β adrenergic blocking agents (DCI-hydergine) and saline constituted one factor. The other factor was composed of saline, *l*-epinephrine, and levarterenol–*dl*-isoproterenol combined. Thirteen replications were made; each replication or block represented an individual experiment with six treatment combinations with one rat per treatment. The random numbers table (17) was used to group the rats into blocks, to determine the sequence of treatments, and to determine the assignment of treatment to each rat. *F* values were calculated at 1 and 55 degrees of freedom and all results indicated as significant are at the 5% level or less (17).

TABLE I.—SUMMARY OF SIX-TREATMENT COMBINATIONS FOR BLOOD SUGAR[a]

Pretreatment	Treatment	Mean Body Wt., Gm.	Mean Blood Sugar Values, mg. %	Mean Deviation from Control	S.E. of the mean[b]
Saline	Saline	230	95	0	± 4.64
Saline	*l*-Epinephrine	226	210	115	±11.75
Saline	Levarterenol–*dl*-isoproterenol	237	138	43	± 7.17
DCI-Hydergine	Saline	232	109	14	± 4.95
DCI-Hydergine	*l*-Epinephrine	251	126	31	± 7.08
DCI-Hydergine	Levarterenol–*dl*-isoproterenol	247	105	10	± 2.75

[a] Thirteen replicates were run on each treatment combination. [b] Significant at the 5% level (17).

TABLE II.—SUMMARY OF SIX-TREATMENT COMBINATIONS FOR BLOOD LACTATE[a]

Pretreatment	Treatment	Mean Body Wt., Gm.	Mean Blood Lactate Values, mg. %	Mean Deviation from Control	S.E. of the Mean[b]
Saline	Saline	230	9.2	0.0	±1.19
Saline	*l*-Epinephrine	226	26.6	17.4	±3.72
Saline	Levarterenol–*dl*-isoproterenol	237	17.5	8.3	±2.57
DCI-Hydergine	Saline	232	14.2	5.0	±2.30
DCI-Hydergine	*l*-Epinephrine	251	15.1	5.9	±2.60
DCI-Hydergine	Levarterenol–*dl*-isoproterenol	247	16.1	6.9	±4.75

[a] Twelve replicates were run on each treatment combination. [b] Significant at the 5% level (17).

RESULTS

Blockade of Catecholamine-Induced Hyperglycemia by Conjoint Administration of Dichloroisoproterenol-Hydergine.—To measure the degree of inhibition, it was of interest to sample only the period during which the maximum increase occurred. According to the reports of Claasen and Noach (4), Ellis (8), and Mayer (5), the maximum output was determined to be approximately 1 hour after intraperitoneal injection. Thus, a sample taken during this period would be most significant in demonstrating the degree of inhibition.

A summary for the six treatment combinations is shown in Table I. These values show that the combined DCI-hydergine is a potent antagonist to the effects of both epinephrine and levarterenol-isoproterenol induced hyperglycemia. The small increase in blood sugar due to the blocking agents alone is approximately 14 mg. %. The subtraction of this value from the values found in treatments (V) and (VI) reflects the true blocking ability of this combination. The adjusted value for treatment (V) is 112 mg. %, which is 17 mg. % above the simultaneous control of treatment (I). This increase is not statistically significant, and it represents an inhibition of 85%. The increase in blood sugar after this treatment combination would be more accurate at 7 mg.%, for the additional 10 mg.% is the result of two observations with large deviations. The degree of inhibition would then be 94%. These values were included as such to maintain the completeness of each block. The rise in blood sugar found in treatment (VI) shows the increase in blood sugar due to the adrenergic blocking agents alone. The values obtained by these two treatment combinations justifies the conclusion that a complete blockade has been demonstrated. The *F* test in the analysis of variance also supports the contention that a complete blockade has been demonstrated.

Blockade of Catecholamine-Induced Hyperlacticacidemia by Conjoint Administration of DCI and Hydergine.—Table II contains a summary of the six treatment combinations. A comparison of treatment (I) and (IV) shows that the blocking agents produced an increase in blood lactate which is statistically significant. Consequently, the observations for treatments (IV), (V), and (VI) had to be adjusted by subtracting this increase, 5 mg. %, before computing the analysis of variance. The similarity of the values found in treatments (IV), (V), and (VI) indicate that the adrenergic blockers have prevented sympathomimetic amine–induced hyperlacticacidemia. By using treatment (IV) as an internal control with 14.2 mg.% as 100% inhibition, the degree of inhibition in treatments (V) and (VI) were 94% and 87%, respectively. The similarity of these three treatment effects and the computed *F* values demonstrated the completeness of this blockade.

DISCUSSION

Because both levarterenol and isoproterenol produced a small but significant increase in blood sugar and because the conjoint administration of these two amines produced an effect greater than either alone, Van der Pol (3) suggested that the glycogenolytic effect was due to the synergistic interactions of both α and β receptors. To confirm this hypothesis, Claasen and Noach (4) studied the glycemic effect of epinephrine, levarterenol, and isoproterenol against the pretreatment of saline and DCI. Their investigation demonstrated that the hyperglycemic effect of levarterenol was unaffected by DCI, while the effect of epinephrine was partially inhibited, and the effect of isoproterenol was completely inhibited. This clearly shows that while the β receptors were blocked, the α receptors continued to mediate glycogenolysis.

Mayer, *et al.* (5), however, took exception to this premise and suggested that the glycogenolytic effect was mediated by the β receptors because (*a*) DCI blocked epinephrine-induced augmentation of cardiac contraction, heart phosphorylase, and hyperglycemia. (*b*) Ergotamine and phenoxybenzamine, both potent α blockers, were unable to prevent the increase in blood sugar and lactic acid. (*c*) DCI partly blocked epinephrine-induced hyperlacticacidemia, but ergotamine had no such effect.

Further evidence for this proposal was advanced by McCutcheon (6) whose investigation of adrenergic amines after adrenergic blockade confirmed those observations made by Mayer, *et al.* (5).

To reconcile the findings of these two groups of investigators, the most obvious means would be to attribute these differences to (*a*) species variation (between dogs and rats), (*b*) differences in route of drug administration (between infusions and subcutaneous injections), or (*c*) the actions of some unknown receptors.

Despite the differences in animals and technique, the results of these two groups can be best explained if the hypothesis is accepted that glycogenolysis is mediated through the interactions of both receptors. Thus, the blockade produced by DCI may be partial because the α receptors were still free to mediate its effects. The partial inhibition of epinephrine-induced hyperlacticacidemia reported by Mayer (5) could also be explained on this basis. Since muscle is the main source of lactic acid and since the receptors here are of the β type (18), this inhibition would be expected.

Inhibition of glycogenolysis with DCI alone has failed to abolish this response completely. This

indifferent antagonism is equally true with the ergot alkaloids. This partial inhibition would be expected if glycogenolysis was mediated through both α and β adrenotropic receptors. If these α and β adrenergic blockers were given together, then the increase in blood sugar and lactic acid can be abolished.

If the contention is true that epinephrine has a greater glycogenolytic effect than either levarterenol or isoproterenol because it stimulates both α and β receptors, then it is reasonable to assume that the combined blockade of both α and β receptors will completely inhibit glycogenolysis. This has been shown to be the case. The results of this study give only an incomplete insight into the mechanisms involved in glycogenolysis, but do suggest that the receptors involved include both the α and the β adrenotropic receptors.

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Polarography of Diastereoisomeric *N*-Nitrosoephedrines

By LOUIS MALSPEIS and NELL G. M. HUNG

The polarographic reductions of *N*-nitrosoephedrine (*erythro* isomer) and *N*-nitrosopseudoephedrine (*threo* isomer) were examined in the pH range 0.6 to 10. Below pH 3.2, the limiting currents of both compounds are diffusion controlled and identical. In the pH range 3.2 to 8.2, the limiting currents of the diastereoisomers are controlled by diffusion and rate of reaction. At a given pH value in this region, under identical conditions, the limiting current for *N*-nitrosoephedrine is greater than that for *N*-nitrosopseudoephedrine. The kinetic currents are catalyzed both by hydrogen ion and general acids. With Delahay's equation, the heterogeneous rate constants of protonation of the diastereoisomers were calculated. The rate of protonation of *N*-nitrosoephedrine is greater than that of *N*-nitrosopseudoephedrine. These relative rates are interpreted in terms of structural considerations. The rate of protonation is greater with that isomer in which there is a lesser degree of intramolecular hydrogen-bonding. Kinetic currents can be used to distinguish between diastereoisomers.

LIMITING POLAROGRAPHIC currents which are controlled by the rate of a chemical reaction and by diffusion are generally referred to as kinetic currents (1, 2). One type of kinetic current is controlled by the rate of reaction in the electrode reaction layer of an electro inactive species which diffuses to the electrode surface from the bulk of the solution. Since the relative reaction rates of diastereoisomers depend upon the population of the possible conformations (3), kinetic currents could be used to distinguish between diastereoisomers. At a given concentration that diastereoisomer which undergoes reaction at the higher rate should afford the higher kinetic current. The primary object of this investigation is to determine the effect of configurational differences on the kinetic currents of diastereoisomers.

The compounds chosen for study are the *N*-nitroso derivatives of (−)-ephedrine and (+)-pseudoephedrine, diastereoisomers having well established configurations (4–7) (see Scheme I). These compounds satisfy the requirement that a center of asymmetry must not be involved in the reaction at the electrode surface, a requirement which increases the likelihood that the reduction mechanisms of the two compounds under comparable conditions will be the same.

EXPERIMENTAL

Materials.—The diastereoisomeric *N*-nitrosoephed-

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