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NEUROTROPIC DRUGS, ELECTROSHOCK, AND CARBOHYDRATE METABOLISM IN THE RAT

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Abstract—The effects of neurotropic drugs on the alterations of blood sugar, lactate, and tissue glycogen levels in relation to blood serotonin level in rats were investigated. Among the drugs employed here, chlorpromazine and chlordiazepoxide were found to increase blood sugar and lactate and to deplete liver glycogen levels in intact rat when administered i.p. On the other hand, in electroshock, hypoglycemia, hyperlactacidemia, and liver glycogen depletion were observed in both intact and adrenodemedullated rats. These changes in electroshock were, to some extent, prevented by the treatment of chlorpromazine and chlordiazepoxide. Electroshock increased the serotonin level in the whole blood of rats and serotonin caused hyperlactacidemia and liver glycogen depletion.

IN 1955, Norman and Hiestand[1] reported a hyperglycemic effect of chlorpromazine (CPZ) in the mouse and hamster, but not in the rat.

Recently, Mayman *et al.*,[2] Mraz and Trinner,[3] and Gey *et al.*[4] have reported an increase of glucose content of the rat brain by the administration of CPZ. Several observations are reported on the alterations of blood sugar, blood lactate, and tissue glycogen levels in the rats treated with CPZ and other tranquilizing agents, as well as the correlation between biogenic amines and carbohydrate metabolism.

The present authors, in previous papers,[5–7] have reported the effects of tranquilizing agents on increases of brain noradrenaline and dopamine levels and decreases of brain serotonin (5HT) level induced by electroshock in rats.

The present study was undertaken to investigate the effects of CPZ and chlordiazepoxide (CDA) on blood sugar, lactate, and tissue glycogen levels of intact and adrenodemedullated rats in relation to blood 5HT level.

MATERIALS AND METHODS

All the rats employed here were male Donryu strain, weighing 180–250 g, maintained on constant diet and water *ad libitum*. The room temperature was kept approximately at 20° throughout the experiment. Rats were fasted for 16–20 hr prior to experiment, with free access to water. Drugs were administered i.p. in doses as follows: CPZ, 20 mg/kg; CDA, 50 mg/kg; imipramine, 25 mg/kg; phenobarbital, 100 mg/kg. They are expressed as free base. Blood samples were withdrawn from a small incision in the tail at intervals of 20, 50, 90, 120, and 180 min after injection.

In the electroshock experiments, pretreatment with CPZ or CDA was carried out 10 min prior to the beginning of electroshock, by the procedure described below; control rats received 0·9% saline. The electroshock with bitemporal electrodes was

performed as follows. Subjecting time was 1 sec in duration with 10-min intervals for 60 min at 20–25 V (A.C.), the threshold stimulus required to produce tonic extensor seizure. The electric current employed in the shock was not measured.

Blood sugar and blood lactate estimations were performed according to the anthrone method[8] and the Barker–Summerson procedure,[9] respectively, after deproteinization with $Ba(OH)_2$ and $ZnSO_4$. In the studies of glycogen, bilateral adrenodemedullation was carried out on rats by the lumbodorsal approach, and no studies were performed earlier than 7 days after the operation. After fasting, rats were fed by the stomach tube with 500 mg glucose/100 g body weight in 50 per cent solution, 3 to 4 hr before subjecting them to electroshock and/or administration of drugs. Rats were sacrificed by decapitation, and tissues were excised as quickly as possible and frozen immediately with solid carbon dioxide. Because of the uneven distribution of glycogen in the rat heart, cardiac glycogen was determined on atrial portions. Two hemidiaphragms and duplicate specimens of hepatic and cardiac tissues were analyzed simultaneously according to the method of Good *et al.*[10] with minor modifications by Kobayashi *et al.*[11]

For the assay of 5HT, animals were sacrificed by decapitation, and bloods were collected into heparinized beakers, transferred to tubes, and centrifuged after deproteinization with $ZnSO_4$ and NaOH. An aliquot of supernatant was employed to assay 5HT in whole blood. Or, brain and liver were quickly removed, blotted with filter paper, and frozen in solid carbon dioxide for later assay. The preparation of homogenate for 5HT assay was carried out in a low-temperature room at 0° with a Potter–Elvehjem-type homogenizer.

In the 5HT experiments, creatinine sulfate salt was used in a dose of 250 μg/rat as the free base. For extraction and estimation of 5HT in tissues, the method described by Bogdanski *et al.*[12] was employed, with the use of a Farrand spectrofluorometer.

The glucose tolerance test was carried out by the method of Christophe and Mayer[13]; that is, fasted rats received glucose i.v. in a dose of 500 mg/kg body weight in 50 per cent solution, and blood samples were withdrawn at 5-min intervals over 30 min; drug-treated rats were injected 30 min prior to the administration of glucose.

RESULTS

Effects of drugs on blood sugar and lactate levels

In intact rats the hyperglycemia and hyperlactacidemia were clearly recognized after administration of CPZ or CDA, but no changes were seen with imipramine and phenobarbital (Fig. 1). As illustrated in Fig. 2, depletion in blood lactate level 2–3 hr after the treatment with CPZ in intact rats was recognized. On the other hand, adrenodemedullation greatly reduced both hyperglycemic and hyperlactacidemic responses with CPZ and CDA and, in addition, significant reduction in blood lactate was observed with CDA.

To investigate the mechanism of hyperglycemia induced by drugs, the glucose tolerance test was performed several times; it was found that tolerance curves of rats treated with drugs were not different from those of controls within 60 min after administration of drugs.

Effects of drugs on tissue glycogen levels

As shown in Table 1, significant reductions of liver glycogen levels were recognized

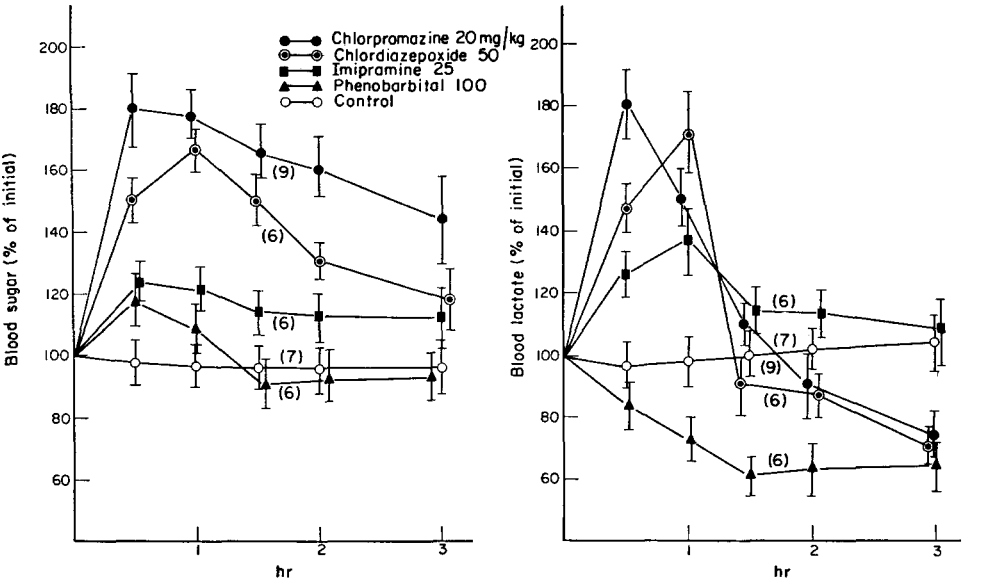

FIG. 1. Effects of neurotropic drugs on blood sugar and blood lactate levels of intact rats. The responses of drugs were investigated over 3 hr after injections.

Throughout the figures in this paper, the following expressions hold: figures in parentheses represent the number of animals employed in each condition (excepted in Fig. 4). The vertical lines indicate standard error of the mean. Drugs were injected i.p.

60 min after the administration of CDA or CPZ, but not imipramine, in adrenodemedullated as well as intact rats. Conversely, clear increase of liver glycogen was observed ($P < 0{\cdot}05$) with phenobarbital. Analysis of variance revealed a highly significant difference between litters in the liver glycogen level; in addition, neither any bias of litter body wt. nor correlation between body weight and response was suggested. It was fortuitous that control values in phenobarbital treatment were lower than other cases. In all cases cardiac and diaphragmatic glycogen levels were not appreciably affected by the treatment of drugs (data not presented here).

Effects of electroshock on blood sugar and lactate levels

With electroshock (20–25 V), the blood sugar levels of rats were depleted to approximately 60 per cent of initial values in both intact and adrenodemedullated rats, but no hypoglycemia was observed with milder stimulation (10 V). In both conditions lactate levels in blood were increased significantly (upper half of Fig. 3). In the pretreatment with CPZ or CDA to investigate the effects of drugs on electroshock, both drugs did prevent the electroshock-induced hyperlactacidemia as well as hypoglycemia (lower half of Fig. 3).

In intact rats, blood sugar levels depleted by electroshock were restored to initial levels by 30 min after the end of electroshock; in contrast, no recovery was found in adrenodemedullated rats. An example of repeated trials was illustrated in Fig. 4 (at left).

As shown in Fig. 4 (middle and right), glucose tolerance curves were made 2, 10,

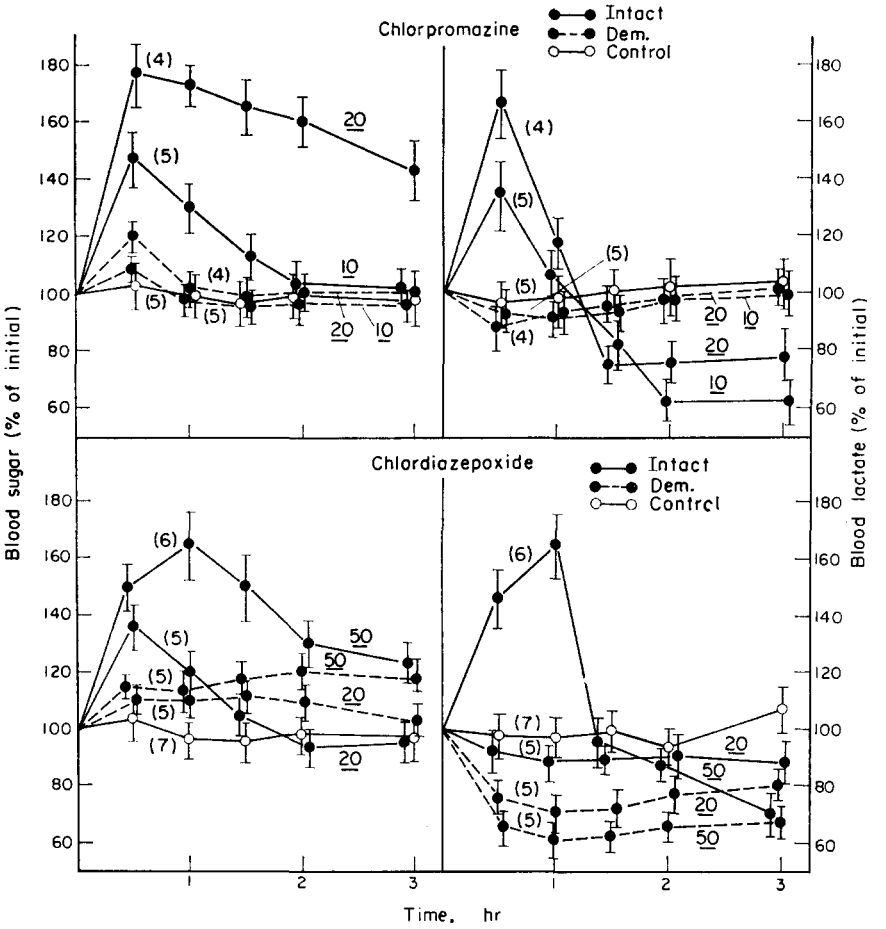

FIG. 2. Effects of chlorpromazine and chlordiazepoxide on blood sugar and blood lactate levels of intact and adrenodemedullated rats. The values with underline represent doses of both drugs. Adrenodemedullation (dem.) was performed more than 7 days before the experiments. Control rats received 0·9 per cent saline.

and 30 min after electroshock; initial peaks (asterisks in the figure) after glucose administration were far lower at 2 and 10 min than at 30 min after the end of electroshock in both intact and adrenodemedullated rats.

Effects of electroshock on tissue glycogen levels

Although electroshock as employed here decreased the liver and diaphragm (adrenodemedullated rats only) glycogen levels in both intact and adrenodemedullated rats, no significant alteration was observed in cardiac glycogen levels (data not presented here).

The diaphragmatic glycogen levels of the treated groups (two litters), $1{\cdot}5 \pm 0{\cdot}14$ (3) and $1{\cdot}4 \pm 0{\cdot}15$ (3), were compared with those of control groups (two litters), $5{\cdot}0 \pm 0{\cdot}13$ (2) and $7{\cdot}5 \pm 1{\cdot}09$ (3) μg/mg tissue. The difference between treated and

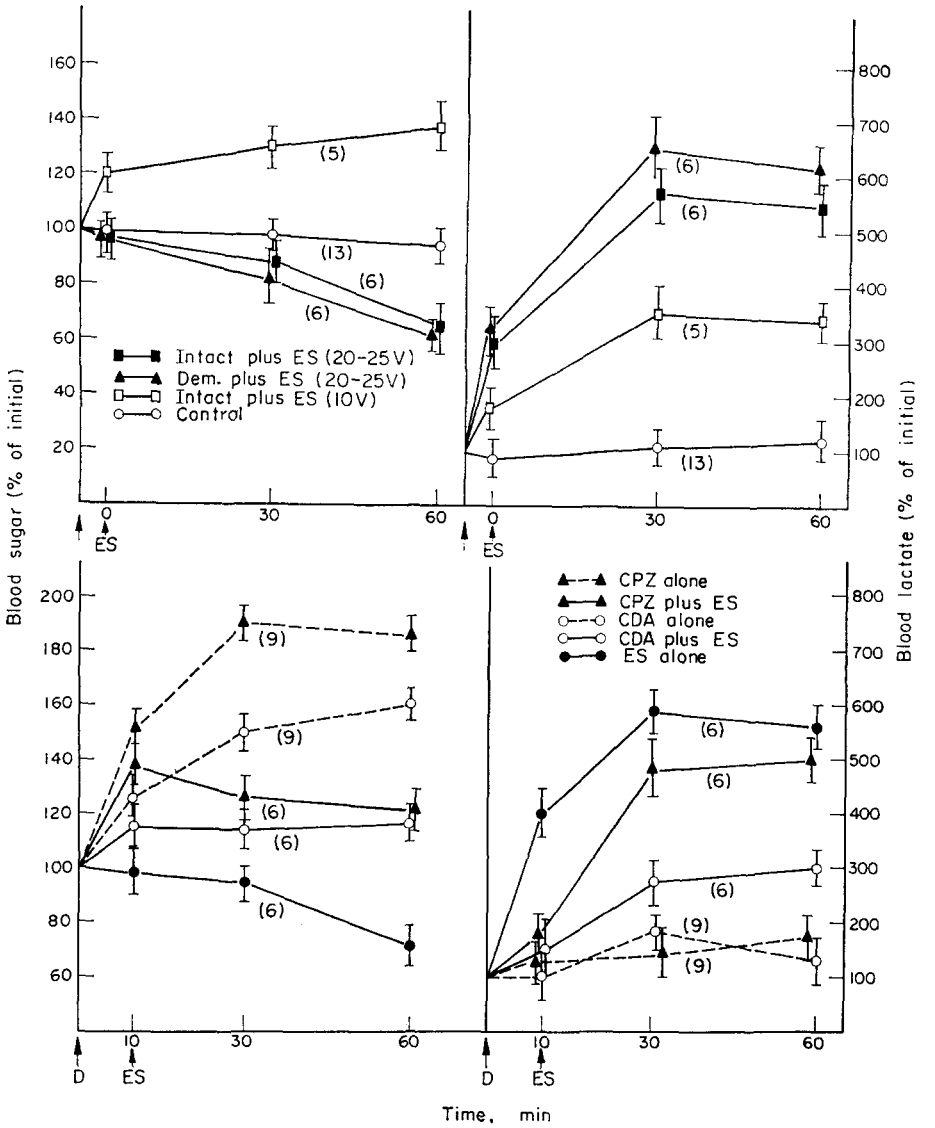

FIG. 3. Effects of electroshock alone and drug plus electroshock on blood sugar and lactate levels of intact and adrenodemedullated (dem.) rats.

Upper: Blood sugar and lactate levels of pretreatment were shown as 100 per cent in the figure indicated by an arrow).

Electroshock: duration, 1 sec/10 min over 60 min, 10 or 20–25 V (A.C.) was used. Electric current in the experiments was not measured. Bitemporal electrodes were employed.

Lower: Doses of chlorpromazine and chlordiazepoxide were 20 and 50 mg/kg respectively. Drugs were injected 10 min before the beginning of electroshock (D point on the figure). Control rats received 0·9 per cent saline.

control rats is statistically significant ($P < 0{\cdot}05$). (Values in parentheses are number of animals used.) Liver glycogen depletion was also obtained by the pretreatment of CPZ, but not with CDA (Table 2).

TABLE 1. EFFECTS OF CHLORPROMAZINE, CHLORDIAZEPOXIDE, IMIPRAMINE, AND PHENOBARBITAL ON LIVER GLYCOGEN LEVELS OF INTACT AND ADRENODEMEDULLATED RATS*

Drug	Condition	Litter No.	Glycogen (μg/mg) T	Glycogen (μg/mg) C
Chlorpromazine (20 mg/kg i.p.)	intact	1	21·4±5·50 (2)	40·3±0·70 (2)
		2	12·3±2·22 (3)	23·0±2·01 (3)
			P<0·05	
	dem.	3	2·0±0·17 (2)	17·1±3·54 (2)
		4	20·0±1·93 (3)	27·2±3·36 (3)
			P<0·05	
Chlordiazepoxide (50 mg/kg i.p.)	intact	5	4·6±1·03 (3)	14·8±1·35 (3)
		6	16·6±0·54 (2)	33·3±0·55 (2)
			P<0·05	
	dem.	7	8·7±0·47 (2)	21·0±3·04 (2)
		8	19·2±3·94 (3)	34·1±1·80 (3)
			P<0·05	
Imipramine (25 mg/kg i.p.)	intact	9	20·5±1·03 (2)	23·0±4·44 (2)
		10	21·4±5·64 (3)	22·8±3·14 (3)
			not sig.	
Phenobarbital (100 mg/kg i.p.)	intact	11	28·6±0·54 (2)	15·6±1·90 (2)
		12	14·4±1·47 (3)	7·9±1·04 (3)
			P<0·05	

* Throughout the tables in this paper each value represents the mean ± standard error of the mean. Figures in parentheses indicate number of animals employed. T and C = treated and control animals; adrenodemedullation (dem.) was performed more than 7 days prior to the experiments. P values are calculated by F-test and compare values of treated groups with those of saline controls.
In this table liver was removed 60 min after drug administration.

To determine whether or not the changes of blood sugar and lactate levels were due to the increase of 5HT in the blood, 5HT was administered s.c. in a dose of 250 μg/rat; results showed increases of blood lactate which were 107 ± 7·48 and 308 ± 7·89 (per cent changes of initial) of levels of control and treated rats respectively. The increase of blood 5HT level and decrease of liver glycogen level 60 min after 5HT injection are shown in Table 3.

Effects of electroshock on tissue 5HT levels

Blood 5HT levels of intact control and treated rats (5 rats each) were found to be 0·26 ± 0·043 and 0·40 ± 0·048 (μg/ml) respectively 60 min after electroshock. These values were statistically significant ($P < 0·05$).

DISCUSSION

The effects of neurotropic drugs on blood sugar, lactate, and tissue glycogen levels have been studied in a number of investigations. Some convulsants depleted the brain glycogen level in mouse,[14] and imipramine treatment in human subjects reduced glucose tolerance.[15] Reserpine-induced hyperglycemia was found by Kuschke and Frantz,[16] who noted no changes of glucose tolerance in rabbit. Gey and Pletscher[17] found reserpine-induced hyperlactacidemia in rat, and Balzer *et al.*[18] showed that his

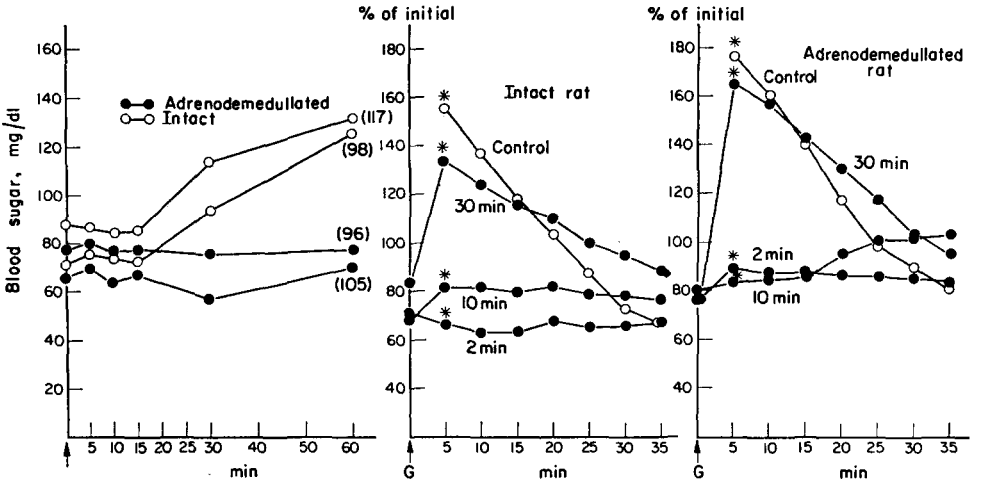

FIG. 4. Blood sugar levels after the end of electroshock, and its effects on glucose tolerance tests in intact and adrenodemedullated rats.

Left: An arrow indicates the blood sugar level immediately after the end of electroshock. Values in parentheses give the blood sugar level (mg/dl) of pretreatment. Blood sugar levels after the end of electroshock were estimated over 60 min. (For mg/dl in this panel *read*: mg/100 ml.)

Middle and right: G point on the graph represents blood sugar levels immediately after the end of electroshock. Rats of three groups were given 50 per cent glucose solution i.v. in the femoral vein in a dose of 500 mg/kg at 2, 10, and 30 min after the end of electroshock, and blood samples were withdrawn at 5-min intervals over 30 min. Control rats (non-electroshock) were also given glucose and treated as were the shocked rats.

TABLE 2. EFFECTS OF ELECTROSHOCK ALONE AND DRUG PRETREATMENT ON LIVER GLYCOGEN LEVELS IN INTACT AND ADRENODEMEDULLATED RATS

Drug	Condition	Litter No.	Glycogen (μg/mg) T	Glycogen (μg/mg) C
None	intact	1	4·9±0·37 (3)	11·5±0·20 (3)
		2	4·2±0·40 (2)	12·1±0·24 (2)
			P<0·05	
	dem.	3	3·7±0·36 (3)	9·4±0·37 (2)
		4	3·5±0·38 (3)	16·5±2·66 (3)
			P<0·05	
Chlorpromazine (20 mg/kg i.p.)	intact	5	6·0±1·15 (3)	16·0±3·00 (3)
		6	3·3±0·68 (3)	20·0±2·01 (2)
			P<0·05	
Chlordiazepoxide (50 mg/kg i.p.)	intact	7	5·0±0·01 (3)	5·3±0·74 (3)
		8	2·3±0·24 (3)	7·5±0·50 (2)
			not sig.	

Electroshock was carried out at 20–25 V (A.C.), 1 sec in duration, with 10-min interval for 60 min. Liver was removed immediately after the end of electroshock.

In the drug pretreatments (litters no. 5–8) T and C indicate drug plus electroshock and drug alone respectively. Drug was given 10 min before electroshock.

TABLE 3. BLOOD SEROTONIN LEVEL AFTER SEROTONIN TREATMENT AND ITS EFFECT ON LIVER GLYCOGEN LEVEL IN INTACT RATS

Treatment	Blood serotonin (μg/ml)		Liver glycogen (μg/mg tissue) 1*	2	
Control	0·34±0·045 (8)	P<0·05	11·1±3·20 (3)	5·3±0·82 (3)	P<0·05
Serotonin	0·58±0·062 (6)		6·7±1·01 (2)	1·7±0·22 (3)	

* Number of littermates. Serotonin was administered s.c. in a dose of 250 μg/rat, and blood and liver were removed after decapitation 60 min later. Control rats received 0·9 per cent saline.

effect was caused in mouse by increase of liver glycogen level owing to an enhanced synthesis of glycogen from non-carbohydrate materials, mediated by the release of catecholamines.

Ryall[19] first observed that CPZ-induced hyperglycemia was associated with decrease of liver glycogen level. Several investigators found that CPZ increased brain as well as blood glucose level and that it markedly reduces liver glycogen level, owing to enhanced utilization by the organism during the first stage of CPZ effect.[20]

On the other hand, Mraz and Trinner[21] and Stoner[22] reported that hyperglycemia may be caused by shock. Others reported that electroshock therapy of patients did not alter the 5HT level in blood of patients,[23] and that brain 5HT levels were reduced with lesions of the central nervous system.[24]

Recently, Moore *et al.*[25] reported the interesting observation that hypoglycemia and liver glycogen depletion occurred in aggregated mice when D-amphetamine was administered in a dose of approximately its LD_{50} value. It is generally recognized that stressful situations cause initial hyperglycemia followed by hypoglycemic response and depletion of tissue glycogen content,[26] however, the initial hyperglycemia seems to fail in some cases.

The results reported in the present paper indicate that chlorpromazine and chlordiazepoxide induce clear increases of blood lactate as well as blood sugar levels in intact rats. Degree of hyperglycemia and hyperlactacidemia was proportional to the increase in dose. The depletions of blood lactate levels induced by phenobarbital 1 hr and by CPZ 2–3 hr after treatment with the drugs (Figs. 1 and 2) may be caused by their sedative action.

Although the results of glucose tolerance tests are, unfortunately, contradictory to the results shown by Jori *et al.*[27] and Bonaccorsi *et al.*,[28] this discrepancy may be due to differences in the procedures used. Consequently it is difficult, at least under the experimental conditions employed here, to assume that CPZ and CDA inhibit the peripheral utilization of glucose.

As pointed out by Jori *et al.*[27] it is doubtless that the integrity of the adrenal medulla is necessary for the onset of hyperglycemia induced by CPZ, as is suggested by the results with adrenodemedullated rats (Fig. 1).

On the other hand, hypoglycemia associated with hyperlactacidemia induced by electroshock in both intact and adrenodemedullated rats was supported by the

glucose tolerance tests. Although lower initial peaks in earlier periods were considerably complicated, the results of glucose tolerance tests were in agreement with the curves of restoring blood sugar levels in electroshocked rats.

It is possible to speculate that electroshock-induced hypoglycemia and liver glycogen depletion are different phenomena having no direct relation to each other. Of considerable interest was the assumption that depletion of blood lactate levels of rats treated with phenobarbital in the present study may have different mechanisms of action from these of CPZ. Further studies, however, are awaited on the mechanism of phenobarbital-induced hypolactacidemia.

In order to evaluate the role of blood 5HT in changes of blood sugar, lactate, and liver glycogen levels, 5HT was injected subcutaneously, and the rise of blood lactate level and liver glycogen depletion were recognized. As reported in our laboratory, 5HT as low as 50 μg/rat caused a vigorous increase of blood lactate level[29] and liver glycogen depletion.[11]

Finally, significant increases of blood 5HT caused by electroshock revealed hypoglycemia and liver glycogen depletion in rats.

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