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Effect of Chlorpromazine and Diazepam on the Metabolic Changes induced by Cold Stress in Rats

A.M. Ageel

Department of Pharmacology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

ABSTRACT. The present investigation was undertaken to study the effects of acute and chronic cold stress on blood glucose, cholesterol, free fatty acids, triglycerides and phospholipids in rats and their modification with chlorpromazine and diazepam. Acute cold stress was produced by exposure of animals to ± 0.5°C for 4 hr. The antianxiety drugs diazepam (2 mg/kg body weight) and chlorpromazine (2 mg/kg body weight) were administered I.P. 30 minutes before the exposure of the animals to cold stress. Immediately after the exposure, the animals were sacrificed and blood samples were collected for biochemical analysis. In chronic studies, the rats were exposed to cold stress (4 ± 0.5°C) for 1 hr daily for six days. Diazepam (2 mg/kg) were given daily 30 minutes before the exposure to cold. On the sixth day, the animals were sacrificed following the exposure and blood was collected for biochemical analysis. The results of this study showed that both diazepam and chlorpromazine tend to normalize the changes in the values of the parameters representing the carbohydrate and lipid metabolism. However, diazepam was comparatively less effective as compared to chlorpromazine in this aspect.

The studies on cold stress have been a centre of interest for biologists for more than three decades. Exposure to low temperatures have proved to be very useful in studying the response to acute and chronic stress (Selye 1952, Le Blanc 1975). It stimulates the sympathetic nervous system as evidenced by a marked increase in epinephrine and norepinephrine secretions (Le Blanc 1969) which in turn produce alternations in carbohydrate and lipid metabolism (Kilburn 1960, Henneman *et al.* 1958, Larson and Ederstorm 1962, Prusiner *et al.* 1968). A large number of drugs have been tried to elucidate the mechanisms of stress-induced changes and to antagonize their harmful effects (Sultanov 1976, Sharma and Parmar 1967, Parmar *et al.* 1967, Tariq 1980).

As the emotional factor plays significant role in the genesis of stress-induced abnormalities (Segal 1981), various anti-anxiety drugs have been investigated for their antistressor activities. The present study investigates the effect of two commonly used tranquilizers, chlorpromazine and diazepam, on cold-stress-induced changes in the carbohydrate and lipid metabolism of rats.

Material and Methods

The present study was undertaken on male albino rats of Wistar strain (8-10 weeks old, 150-200 g body weight) fed on standard rat chow diet and water *ad libitum*. A light cycle of 10 hr in dark and 14 hr in light was maintained and the animals were allowed to become acclimated to the laboratory conditions (ambient temperature of 25°C) for one week. Thereafter, the animals were randomly divided into groups of 10 rats as follows:

Control (Normal)

These animals received only normal saline (1 ml/kg body weight, i.p., once) and were not exposed to any stress.

Control Acute Stress

Acute stress was produced in rats by exposing the animals to a temperature of 4 ± 0.5°C for a period of 4 hr. These animals received 1 ml/kg body weight i.p. of normal saline or propylene glycol, 30 min prior to cold exposure.

Chlorpromazine + Acute Stress

The animals were pretreated with 2 mg/kg body weight of chlorpromazine i.p. 30 min before the cold exposure.

Diazepam + Acute Stress

The animals in this group were treated with diazepam, 2 mg/kg body weight i.p. 30 min before the cold exposure.

Control Chronic Stress

The rats were subjected to cold stress at 4 ± 0.5°C for 1 hr daily for six consecutive days. Animals in this group were also treated with normal saline or propylene glycol 1 ml/kg body weight i.p. 30 min before each exposure to cold.

Chlorpromazine + Chronic Stress

The animals in this group were treated with chlorpromazine, 2 mg/kg body weight, i.p. 30 min before each exposure to cold as described above.

Diazepam + Chronic Stress

In this group the animals were pretreated with diazepam, 2 mg/kg body weight i.p., 30 min before each exposure to cold as described above.

Drugs

Chlorpromazine HC1 (May & Baker) and diazepam (Roche Inc.) were used. Chlorpromazine was administered dissolved in normal saline whereas diazepam was dissolved in propylene glycol. The volume of all the injections was kept constant at 1 ml/kg.

Biochemical Analysis

Immediately after exposure, the animals were sacrificed using anaesthetic ether and blood samples were immediately collected from the heart for the estimation of glucose (Torlotin 1966), cholesterol (Glick 1963), nonesterified fatty acids (NEFA) (Duncombe 1964) triglycerides (Bergmeyer 1975) and phospholipids (Zilversmith 1950). The statistical analysis of data was done using Student's 't' test.

Results

The results of this study have been summarized in Table 1. The administration of the vehicles used (*i.e.*, normal saline or propylene glycol) did not produce any significant alteration in the values of the parameters studied. The control groups indicate the values of saline pretreated animals.

Blood Glucose

The mean blood glucose value of the normal rats was 82.80 ± 1.04 mg/100 ml. When the animals were subjected to acute cold stress there was a slight but significant increase in the blood glucose. It was, however, highly significant in the chronically stressed rats. Pretreatment with diazepam further accentuated the rise in blood glucose in the acute stressed group, though it was statistically insignificant, whereas both drugs tend to produce a significant reduction of the hyperglycemia of the chronically stressed animals.

Serum Cholesterol

The mean serum cholesterol value of the normal rats was 40.10 ± 0.75 mg/100 ml., which significantly increased after subjecting the animals to acute and chronic cold stress. The increase in serum cholesterol followed the same pattern as did blood glucose under similar stressed conditions. Pretreatment with chlorpromazine led to a highly significant reduction of serum cholesterol in the animals subjected to acute cold and the readings reached a hypocholesterolemic level. Although the reduction in the chronically stressed animals was significant, the level was in the hypercholesterolemic range. Treatment with diazepam decreased only the cholesterol levels of rats subjected to chronic stress.

Table 1. Effect of chlorpromazine and diazepam on carbohydrate and lipid metabolism of rats subjected to acute and chronic cold stress.

Treatment	Blood Glucose (mg/100 ml) ± S.E.	Cholesterol (mg/100 ml) ± S.E.	Nonesterified fatty acids (m Eq/1 ± S.E.)	Triglycerides (mg/100 ml) ± S.E.	Phospholipids (mg/100 ml) ± S.E.
Control (Normal)	82.80 ± 1.04	40.10 ± 0.75	0.078 ± 0.003	92.75 ± 1.04	177.68 ± 2.04
Acute Stress					
Stress	177.69 ± 5.90*	60.46 ± 2.00**	0.112 ± 0.007*	89.00 ± 1.92	209.29 ± 3.50*
Stress + Chlor-promazine (2 mg/kg)	110.60 ± 2.44	32.50 ± 1.22***	0.078 ± 0.012**	48.80 ± 1.38**	174.70 ± 2.90**
Stress + Diaze-pam (2 mg/kg)	139.20 ± 8.50	68.50 ± 4.03	0.095 ± 0.008	62.20 ± 1.55**	165.40 ± 2.70**
Chronic Stress					
Stress	234.00 ± 5.72***	118.60 ± 3.46***	0.496 ± 0.006***	160.20 ± 5.60***	210.40 ± 4.85*
Chlorpromazine Pretreatment	148.30 ± 2.53***	97.50 ± 2.80**	0.105 ± 0.002***	65.60 ± 3.16***	151.90 ± 2.80***
Diazepam Pretreatment	180.56 ± 5.51**	105.44 ± 2.68**	0.289 ± 0.004***	73.33 ± 2.35***	192.44 ± 2.84**

* $P < 0,05$, ** $P < 0.01$, *** $P < 0.001$ Students 't' test.

The values of the stressed groups were compared with those of the control (normal) values and the values in the groups treated with the drugs were compared with those of the corresponding stressed groups.

Nonesterified Fatty Acids (NEFA)

The mean normal NEFA value was found to be 0.078 ± 0.003 mEq/*l* in rats. The rise in the NEFA levels in the acute and chronically stressed animals followed the same pattern as did blood glucose and serum cholesterol. Treatment with chlorpromazine almost normalized the increase of NEFA in both the experimental groups, whereas diazepam was not effective in altering the increased NEFA contents in acutely stressed animals and the decrease of NEFA levels in chronically stressed group although significant, was not comparable to that of chlorpromazine treated rats.

Serum Triglycerides (TGs)

The mean normal serum TGs value of rats was found to be 92.75 ± 1.04 mg/100 ml. The serum TGs did not show any change in the acutely stressed animals but registered a significant increase in the chronically stressed group. Both chlorpromazine and diazepam led to reduction of TGs levels in the acute and chronic groups and the levels after treatment with these drugs were considerably below the normal levels.

Serum Phospholipids

The mean serum phospholipids value of normal rats was 177.6 ± 2.04 mg/100 ml which showed a slight but significant increase when the animals were subjected to acute or chronic cold stress. Pretreatment with chlorpromazine and diazepam significantly reduced the elevated phospholipids of the acute and chronically stressed rats.

Discussion

An intimate correlation between the carbohydrate and lipid metabolism and normal physiological activities of the body has been proposed by many workers. In the present study, both the acute and chronic exposure to cold resulted in almost similar changes in the parameters representing the carbohydrate and lipid metabolism. The changes produced were more profound in the chronically stressed groups except those seen in the phospholipid levels.

Cold stress induced hyperglycemia was in agreement with that reported by a majority of workers (Henneman *et al.* 1958, Kilburn 1960, Larson and Ederstrom 1962). This hyperglycemic activity can be attributed to various factors including release of epinephrine during stress (Kilburn 1960), the decreased utilization of glucose, defective storage of glycogen in the liver, failure to convert glucose to glycogen (Fuhrman and Fuhrman 1963), increased formation of glucose from the liver glycogen (Bickford and Mottram 1960), increased rate of intestinal absorption during short exposure to cold (Cordier and Piery 1950) and the decreased glomerular filtration of glucose (Kanter 1959). In the present study, both chlorpromazine

and diazepam have been found to significantly antagonize the hyperglycemia only under chronic conditions. Chlorpromazine possesses significant antiadrenergic activity (Deshpande and Tiwasker 1960) in addition to its tranquilizing effect but diazepam is devoid of any anti-adrenergic activity.

It appears that the antiadrenergic activity of chlorpromazine may be responsible for prevention of stress-induced hyperglycemia. The reduction of blood glucose in the chronically stressed rats by diazepam may be attributed to the decreased turnover of norepinephrine in the brain (Gilman *et al.* 1980) which may in turn lead to decreased sympathetic activity. The failure of chlorpromazine in reducing the hyperglycemia under acute conditions can be attributed to its own initial stimulant effect on the adrenal gland (Vogt 1954, Mraz and Triner 1963). The blood glucose level in the acutely exposed diazepam treated animals was higher than that of acutely exposed control animals, however, the difference between them was not found to be statistically significant.

The lipolytic actions seen under stress conditions have been attributed to the increased levels of norepinephrine (Prusiner *et al.* 1968). The increases in blood cholesterol, NEFA and phospholipids under acute cold exposure and the increase in the blood triglycerides along with the above under chronic exposure to cold stress are similar to the earlier reports (Maickel *et al.* 1961, Havel *et al.* 1963, Tariq 1980). The increase in plasma NEFA concentration has also been reported by Lorentzen (1964) in man and by Chiodi and Bass (1969) and Jones *et al.* (1972) in rats. Such increase in NEFA is usually attributed to a direct action of catecholamines on adipose tissues (Carlson and Bally 1965). The increase in heat production on exposure to cold is an important means to maintain body temperature. When the loss of body heat is accelerated by exposure to low environmental temperature, additional fuel must be made available to cope with increased demand for energy. It is believed that lipids are the most important fuel for oxidation in this context (Carlson and Bally 1965). Therefore, immediate and marked increase in blood levels of NEFA is an invariable response to severe cold exposure in man (Wilson *et al.* 1969) and animals (Blatties 1966).

The exposure to chronic stress also produced similar metabolic changes as seen after the acute stress. The serum TGs levels, which did not change during acute exposure, also registered a significant elevation after chronic exposures. The changes in all the parameters, except the phospholipids, were more profound as compared to those observed after acute stress. Chlorpromazine pretreatment led to significant reduction of serum cholesterol in both groups, whereas diazepam caused a significant reduction of serum cholesterol only in the chronic stress group. On the other hand, it tended to raise the cholesterol in acutely stressed group. However, this increase was not significant. Chlorpromazine produced similar normalizing effect on the other parameters of lipid metabolism included in this study. Diazepam was less effective as compared to chlorpromazine in general, and it also

failed to antagonize some of the changes induced by the acute stress, *e.g.*, blood glucose, serum cholesterol and NEFA. While the protective effect of chlorpromazine can be explained by its tranquilizing and antiadrenergic activities, the lesser efficacy of diazepam can be attributed to the absence of antiadrenergic activity. The recent report of Odio and Maickel (1982) on the protective effect of diazepam on cold stress induced increase in plasma free fatty acids in rats are also in corroboration with our results and conclusions.

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تأثير الكلوربرومازين والديازيبام على التغيرات الأيضية المسببة بالضغط النفسي الناتج من تعرض الجرذان للبرودة

عبد الرحمن محمد عقيل

قسم علم الأدوية - كلية الصيدلة - جامعة الملك سعود - الرياض - المملكة العربية السعودية

لقد أجريت الدراسة الحالية للتعرف على مدى التأثير الذى يحدثه الضغط النفسي الواقع على الجرذان سواء وقتياً أو على فترة زمنية ممتدة وذلك على معدلات الدم للجلوكوز والكوليسترول والأحماض الدهنيةالحرة والجليسريدات الثلاثية والدهنيات الفوسفورية وأيضاً لمعرفة التغيرات التى تطرأ على هذه المعدلات إذا ما استعمل الكلوربرومازين والديازيبام قبل إحداث الضغط النفسي. طريقة إحداث الضغط النفسي الوقتي تمت بتعريض الحيوانات لدرجة حرارة تبلغ ٤ ± ٠,٥ درجة مئوية لمدة أربع ساعات. وكانت الأدوية المانعة للقلق المستخدمة في هذه الدراسة، وهى الكلوربرومازين والديازيبام، تحقن بجرعة تبلغ ٢ مجم لكل كيلو جرام من وزن الحيوان عن طريق التجويف البريتوني قبل تعريض الحيوان للبرودة بفترة زمنية تبلغ نصف ساعة. أما الطريقة التى اتبعت لإحداث الضغط النفسي المزمن فكانت عن طريق تعريض الحيوانات لنفس درجة الحرارة السابقة ولكن لمدة ساعة يومياً طيلة ستة أيام كان يتم خلالها حقن الكلوربرومازين والديازيبام (٢ مجم/كيلوجرام من

وزن الحيوان) يومياً وذلك قبل تعريض الحيوانات للبرودة بنصف ساعة. في كلتا الطريقتين كانت الحيوانات تقتل بعد الانتهاء من التجربة مباشرة، وكانت عينات الدم تُجمع لإجراء التحاليل اللازمة.

أظهرت النتائج المستخلصة أن تعرُّض الجرذان لظروف البرودة السابقة أدى إلى تغيير في معدلات الدم للمواد التى تم تقديرها في حين أن استخدام الكلوربرومازين والديازيبام أعاد هذه المعدلات إلى مستواها الطبيعي في الدم إلا أن فعل الديازيبام كان أقل تأثيراً من فعل الكلوربرومازين.