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CHLORPROMAZINE-INDUCED HYPOTHERMIA AND INCREASED PLASMA CREATINE PHOSPHOKINASE ACTIVITY*

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Abstract—The rate of efflux of creatine phosphokinase (CPK) from rat extensor digitorum longus muscle *in vitro* was significantly less at 22° than at 30°. Chlorpromazine (Cpz) at 10^{-3} M and 10^{-4} M, but not at 10^{-5} M increased the efflux of CPK *in vitro*. *In vivo*, Cpz produced levels of CPK in plasma which were significantly correlated with the hypothermia that developed after the drug was administered ($R = -0{\cdot}958$, $P < 0{\cdot}001$). When the hypothermia subsequent to Cpz administration was blocked by keeping rats at 31° or by administering the drug to cold acclimated rats, no increase in plasma CPK levels developed. Cpz, 25 mg/kg, produced a greater fall in body temperature and a greater increase in plasma CPK activity in rats kept at 2° than in rats kept at 22°. Cpz, given intramuscularly (i.m.) or intraperitoneally (i.p.) to rats kept at 22° produced equivalent increases in plasma CPK activity although the hypothermia following i.m. administration was slightly greater. Ganglionic blockade did not inhibit the increase in plasma CPK levels. Adrenalectomized rats did not differ from intact rats in the extent of increase in plasma CPK levels for a given degree of hypothermia. It is suggested that the increased plasma levels of CPK *in vivo* following Cpz in rats is due to the hypothermia produced by this drug rather than a direct toxic effect. In man, where IM Cpz does not produce hypothermia, the increased levels of CPK in plasma are probably due to the toxic effects of the drug, or its vehicle, on muscle.

INCREASED activity of the muscle isoenzyme of creatine phosphokinase (CPK) (EC 2.7.3.2) has been found in 60–70 per cent of acutely psychotic patients at the onset of a psychotic episode.[1,2] The effects of phenothiazine medication on the activity of this enzyme was of interest, therefore, since many such patients are treated with phenothiazines. Orally administered phenothiazines were not found to produce increased CPK activity in man.[1] Warnock and Ellman attributed the increased serum CPK activity in some psychotic patients to the effects of intramuscular chlorpromazine (Cpz).[3] In proof of their hypothesis, they showed that intramuscular (i.m.) injections of Cpz in rabbits (4·2–6·2 mg/kg) produced substantial increases in serum CPK activity of the muscle-type isoenzyme.[3] In one study, 16 of 25 (64 per cent) acutely psychotic patients who had not had psychotropic medication of any kind had increased serum CPK activity so that i.m. chlorpromazine could not be the cause of the increases in all acutely psychotic patients.[4] We demonstrated that 6 of 14 (42 per cent) of the humans injected with Cpz i.m. (0·5–1·0 mg/kg) had increased CPK activity.[5]

The increase in plasma CPK activity in man and rabbits was attributed to a local toxic effect of Cpz on muscle at the injection site by Warnock and Ellman.[3] Toxic

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effects of Cpz were also cited by Gielen as the explanation of increases in the activity in serum of aspartate aminotransferase (SGOT), lactic dehydrogenase, malic dehydrogenase and isocitric dehydrogenase following the intraperitoneal (i.p.) injection of Cpz, 5 mg/kg, in rats.[6]

Hypothermia in animals is associated with increased activity of some serum enzymes.[7–10] Since Cpz produces hypothermia,[11] we decided to explore the possibility that the enzyme release following Cpz might be secondary to the hypothermia induced by Cpz. The role of catecholamines in the increase in CPK activity, which occurs following Cpz, was also explored since Cpz increases the excretion of norepinephrine and epinephrine and an intravenous infusion in dogs of norepinephrine (0·5–0·8 mg/kg) or epinephrine (0·55–0·9 mg/kg) increased SGOT activity.[12,13] The increased secretion of norepinephrine following Cpz is mainly derived from sympathetic nerve endings.[12] However, the release is a centrally mediated response, as it is prevented by the ganglionic blocker mecamylamine.[12]

It is also of interest to study the role of adrenocorticoids in the increased plasma CPK activity following Cpz. Cpz has been reported to stimulate the adrenal–pituitary–hypothalamic system.[14] Cortisone increases the plasma aldolase activity in the rabbit,[15] while adrenalectomy blocks the increase in SGOT activity which is produced by restraint stress in rats.[16] We also considered the possibility that the increased activity of plasma enzymes following Cpz is due to diminished clearance of CPK from plasma.

METHODS

In vitro *studies*

Extensor digitorum longus muscles were rapidly dissected from both legs of male Sprague–Dawley rats which had been decapitated. The muscles were kept moist with the tris-buffered medium described by Dawson[17] which was modified to contain 100 mg per cent bovine serum albumin and cysteine HCl, 10^{-3} M.* The final pH was 7·5. Ties made of surgical cotton were placed at each end of the muscle and attached to a C-shaped plastic bar so that the muscle was gently stretched. Each muscle tied to a bar was placed in a chamber containing 15 ml of the medium. The incubation was in air in a Dubnoff metabolic shaker at 120 cycles/min. The muscle was always completely covered with medium. 0·2-ml aliquots were removed at the onset of an incubation and at 30, 60 and 120 min thereafter. The CPK activity of the aliquot was determined immediately by the method of Rosalki.[18] Aliquots with activity greater than 250 mU/ml were diluted with normal saline.

Incubations were conducted at a bath temperature of 22° or 30° for 2 hr. In some experiments with a bath temperature of 30°, Cpz was added to a final concentration of 10^{-3} M, 10^{-4} M or 10^{-5} M. The pH was readjusted to 7·5 if necessary.

In vivo *studies*

Male Sprague–Dawley rats, 125–150 g, were utilized throughout these experiments, except where indicated. All experimental and control groups consisted of six rats, except where indicated. The rats were housed in groups of six at 22° and had free access to food and water *ad lib.* For the experiments with cold acclimitization, rats were

* These modifications were made at the suggestion of Dawson (personal communication) in order to minimize loss of activity of CPK in the medium through denaturation.

housed individually at 2° for 21 days with access to food and water *ad lib*. Adrenalectomized male Sprague–Dawley rats, 125–150 g, were obtained from Hormone Assay Labs, Chicago, Illinois, along with unoperated rats of the same strain. They were given food and saline *ad lib*. and were studied 1 week after surgery. For the study of the effects of ambient temperature on plasma CPK activity, rats which had been kept at 22° were placed in a cold room (2°) for 2 hr, or an incubator (31°), in indivudual cages, for 4 hr following Cpz or saline administration.

Cpz was dissolved in saline for administration and all control animals received saline. The volume of solution administered was 0·1 ml. Intramuscular injections were given into the anterior thigh with a 26 gauge needle.

To compare Cpz and Thorazine®, injections of each preparation were administered i.m. as described above.

Body temperature was monitored with a Tele-thermometer (Yellow Spring Instrument Co.) using a small animal probe inserted 4 cm into the rectum. To terminate an experiment, the animal was anesthetized with pentobarbital i.p. (200 mg/kg) after temperature determination and heparinized plasma was obtained from the inferior vena caga. Plasma CPK activity was determined by the method of Rosalki.[18] The paired *t*-test was used to analyse the statistical significance of differences in plasma CPK activities and rectal temperatures.

RESULTS

In vitro *studies*

The rate of efflux of CPK from rat extensor digitorum longus *in vitro* was significantly less at 22° than at 30°. Cpz, at a concentration in the medium of 10^{-3} M and 10^{-4} M, but not at 10^{-5} M, significantly increased the rate of efflux of CPK from this muscle *in vitro* at 30° (Fig. 1). The statistical significance of these differences is indicated in Fig. 1.

TABLE 1. TEMPORAL RELATIONSHIP BETWEEN PLASMA CPK ACTIVITY AND RECTAL TEMPERATURE FOLLOWING i.p. Cpz AT 22°

Time of sacrifice (hr)	Rectal temp. ± S.D.† (°)	Plasma CPK activity ± S.D.† (mU/ml)
0*	38·5 ± 0·2	61 ± 13
1/2	36·5 ± 0·8	197 ± 11
1	36·0 ± 0·9	381 ± 223
2	35·0 ± 1·1	425 ± 202
3	34·3 ± 1·0	487 ± 164
4	33·6 ± 0·5	607 ± 124
8	36·0 ± 0·4	263 ± 106
24	37·1 ± 0·1	94 ± 30

Groups of six animals received Cpz (25 mg/kg), i.p. and were sacrificed at various times thereafter.

* The control group consisted of 65 rats.

† Product-moment correlation = −0·958, $P < 0·001$.

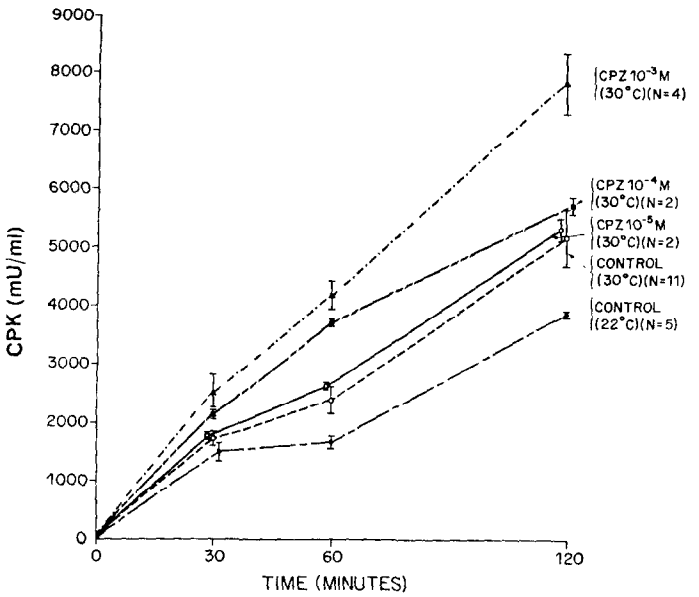

FIG. 1. Effect of bath temperature and Cpz on efflux of CPK from rat extensor digitorum longus *in vitro*. Extensor digitorum longus muscles were dissected from male, 150 g Sprague–Dawley rats. The muscles were tied to supports via the tendons and incubated for 120 min as described under Methods, at 22° or 30°, with or without Cpz. CPK activity in the medium was analysed immediately as described under Methods. The significance of the difference of the efflux from the efflux of the control at 30° was analysed by means of a two-tailed *t*-test and is given below:

Time (min)	P value			
	> 0·05	< 0·05	< 0·005	< 0·001
30	Cpz-10^{-5} M	Control-22°	Cpz-10^{-4}	Cpz-10^{-3} M
60	Cpz-10^{-5} M			Control-22°
				Cpz-10^{-3} M
				Cpz-10^{-4} M
120	Cpz-10^{-4} M			Control-22°
	Cpz-10^{-5} M			Cpz-10^{-3} M

In vivo *studies*

Temporal relationship between hypothermia and plasma CPK activity. CPK activity and rectal temperature at various intervals after Cpz administration were highly inversely correlated (Table 1): $r = -0{\cdot}958$, $P < 0{\cdot}001$. The peak increase in plasma CPK activity occurred at the same time as the lowest body temperature, i.e. 4 hr after Cpz. However, the greatest rate of change in plasma CPK activity occurred within 30 min after Cpz, during which temperature fell only 0·9°.

In another group of 12 rats kept at a room temperature (22°), and then sacrificed 4 hr after receiving Cpz (25 mg/kg i.p.), the Spearman rank-order correlation between rectal temperature and plasma CPK activity was $r = -0{\cdot}866$, $P < 0{\cdot}01$ (Table 2).

Effect of i.p. Cpz at 2° (Table 3). Rats given Cpz (25 mg/kg) and kept at 2° for 4 hr had a very profound fall in rectal temperature and large increases in plasma CPK

activity, both of which were significantly greater than those in rats which were given Cpz and kept at 22°. Rectal temperature decreased slightly and plasma CPK activity increased slightly in saline-treated rats expos ' to 2° for 2 hr in individual cages.

Effect of i.m. and i.p. Cpz at 22° (Table 3). our hr after Cpz (25 mg/kg), marked hypothermia and significant increases in plasma CPK activity were present in rats at an environmental temperature of 22° regardless of whether the Cpz was administered i.m. or i.p. The rats who received Cpz i.m. had a slightly greater degree of hypothermia than the rats which received it i.p. The plasma CPK activity after i.m. injections was also somewhat greater, but the difference was not significant. As reported by Dundee *et al.*[19] we noted no shivering in the Cpz treated animals despite the marked hypothermia.

TABLE 2. CORRELATION BETWEEN PLASMA CPK ACTIVITY AND RECTAL TEMPERATURE 4 hr AFTER Cpz

Animal	Plasma CPK activity (mμ/ml)	CPK rank order	Rectal temp. (°)	Temp. rank order
1	632	2	33·0	1
2	744	1	33·5	2
3	444	4	34·2	4
4	308	10	35·8	10
5	516	3	35·0	5
6	356	6	35·2	6·5
7	252	11	36·2	11
8	348	7	33·9	3
9	332	8	35·7	9
10	116	12	36·5	12
11	312	9	35·2	6·5
12	376	5	35·3	8

Twelve rats were given Cpz (25 mg/kg), i.p. at 22°, and sacrificed at 4 hr, after taking their rectal temperature. Spearman rank order correlation $= -0{\cdot}866$, $P < 0{\cdot}01$.

Effect of i.m. Cpz and Thorazine at 22°. Four hr following the injection of Cpz (10 mg/kg), dissolved in saline, plasma CPK activity was 136 ± S.D.40 mU/ml. This was not significantly different from the plasma CPK activity after an i.m. injection of the same dose of Thorazine 153 ± S.D. 35 mU/ml using a paired *t*-test. Each group consisted of six animals.

Effect of i.m. and i.p. Cpz at 31° (Table 3). Rats kept at 31° following Cpz (25 mg/kg), i.m. or i.p. demonstrated significant inhibition of hypothermia ($P < 0{\cdot}001$) and significantly smaller increases in plasma CPK activity in comparison with rats given Cpz at 22° ($P < 0{\cdot}001$). There was no significant difference in plasma CPK activity between rats given Cpz or saline i.m. or i.p., at 31°. Rats given Cpz i.m. at 31° had a slightly higher temperature than rats given saline i.m. at 31° while the reverse was true for the two groups when the injections were given i.p. If rats were given Cpz at 22° and 1 hr after, put in a 31° incubator, the mean rectal temperature and the mean plasma CPK activity at 4 hr were normal even though a significant hypothermia was present at 1 hr (Table 4).

TABLE 3. EFFECT OF AMBIENT TEMPERATURE AND COLD ACCLIMATION ON RESPONSE OF PLASMA CPK ACTIVITY AND RECTAL TEMPERATURE

Route	Treatment	Plasma CPK activity ± S.D. (mU/ml) Normal rats	Rectal temp. ± S.D. (°)
i.p.	Cpz, 2°	1075 ± 158[1]*	11·0 ± 0·1[13]‡
	22°	393 ± 40[2]	34·8 ± 0·4[14]
	31°	107 ± 3[3]	37·8 ± 0·3[15]
	Saline, 2°	106 ± 17[4]	37·6 ± 0·3[16]
	22°	61 ± 13[5]†	38·5 ± 0·2[17]
	31°	103 ± 12[6]	37·3 ± 0·2[18]
i.m.	Cpz, 22°	457 ± 83[7]	33·3 ± 0·9[19]
	31°	109 ± 20[8]	37·6 ± 0·1[20]
	Saline, 22°	69 ± 10[9]	38·0 ± 0·2[21]
	31°	106 ± 6[10]	37·3 ± 0·2[22]
		Cold acclimated rats§	
i.p.	Cpz, 22°	144 ± 14[11]	37·1 ± 0·0[23]
	Saline, 22°	80 ± 6[12]	37·8 ± 0·2[24]

* Each value represents the mean of six animals at the end of the experiment, except where noted below. All animals were sacrificed 4 hr after Cpz or saline administration.

† This represents the mean value of 65 rats.

‡ Mean of four animals; two died.

§ The rats were cold acclimated for 21 days. Following Cpz or saline administration, they were kept at 22° for 4 hr.

P values were determined using a paired *t*-test in comparing the values between groups of equal size. For tests of significance involving groups of unequal size, the unpaired *t*-test was used, with correction for unequal variances where appropriate.

P values

> 0·10	< 0·05	< 0·01	< 0·025	< 0·005	< 0·001	
[2] vs. [7]	[9] vs. [10]	[14] vs. [19]	[15] vs. [18]	[7] vs. [8]	[1] vs. [2]	[13] vs. [16]
[3] vs. [6]			[20] vs. [22]	[19] vs. [20]	[1] vs. [3]	[14] vs. [15]
[3] vs. [8]			[21] vs. [22]		[1] vs. [4]	[14] vs. [16]
[8] vs. [10]					[2] vs. [3]	[14] vs. [17]
					[2] vs. [5]	[14] vs. [23]
					[2] vs. [11]	[14] vs. [24]
					[2] vs. [12]	[17] vs. [18]
					[5] vs. [6]	[23] vs. [24]
					[13] vs. [14]	

Effect of cold acclimation on plasma CPK activity following Cpz (Table 3). Cold acclimated rats given Cpz i.p. and kept at 22° for 4 hr thereafter had a minimal fall in rectal temperature and a significantly smaller increase in plasma CPK activity than naive rats given the same dosage of Cpz. However, the fall in rectal temperature and the plasma CPK activity of the Cpz-treated cold acclimated rats were significantly greater than saline-treated cold acclimated rats.

Effect of adrenalectomy and cortisone of plasma CPK activity and rectal temperature. Adrenalectomized rats had no greater increase in plasma CPK levels 4 hr following Cpz administration than did controls, and an equivalent temperature fall. However,

TABLE 4. EFFECT OF 31° AMBIENT TEMPERATURE ON RECTAL TEMPERATURE AND PLASMA CPK ACTIVITY IN RATS GIVEN Cpz 1 hr PREVIOUSLY

Environment	Treatment (mg/kg)	Plasma CPK activity (mU/ml ± S.D.)	P	Rectal temperature (°) ± S.D.	P
22°, 4 hr	Cpz-25, i.p.	393 ± 40	—	34·8 ± 0·4	—
22°, 1 hr 31°, 3 hr	Cpz-25, i.p.	84 ± 8	0·001	38·1 ± 0·4	0·001

Two groups of six rats each were given Cpz 25 mg/kg, i.p. One group was kept at 22° for 4 hr; the other was kept at 22° for 1 hr and then put into a 31° incubator, for 3 hr. Data was analysed using a paired *t*-test.

TABLE 5. EFFECT OF ADRENALECTOMY ON RESPONSE TO Cpz AT 22°

Subjects	Plasma CPK activity ± S.D. (mU/ml)	P	Rectal temperature ± S.D. (°)	P
		4 hr		
Adrenalectomized	289 ± 45		34·2 ± 0·4	
Controls	357 ± 71	N.S.	35·1 ± 0·3	N.S.
		8 hr		
Adrenalectomized	509 ± 43		29·9 ± 1·3	
Controls	263 ± 106	< 0·001	36·0 ± 0·4	< 0·001

Each group of six animals received Cpz, 25 mg/kg i.p. Two groups were left at 22° for 4 hr and two for 8 hr. All data was analysed using a paired *t*-test.

TABLE 6. EFFECT OF CORTISONE, EPINEPHRINE AND NOREPINEPHRINE ON PLASMA CPK ACTIVITY AT 22°

Route	Drug	Dose (mg/kg)	Number of animals	Plasma CPK activity ± S.D. (mU/ml)	P*
i.p.	Saline	—	65	61 ± 13	—
i.p.	Cortisone	5	6	59 ± 9	N.S.
s.c.	Epinephrine	1·25	12	1570 ± 600	< 0·001
s.c.	Norepinephrine	4	6	260 ± 71	< 0·001

* The data was analysed using an unpaired *t*-test with correction for unequal variances where appropriate.

at 8 hr, the adrenalectomized rats had a greater temperature fall than control rats and higher plasma CPK levels (Table 5). Cortisone (5 mg/kg, i.p.), produced no change in plasma CPK activity 4 hr after drug administration (Table 6).

Effect of hexamethonium, epinephrine and norepinephrine on plasma CPK activity and rectal temperature following Cpz. The mean fall in rectal temperature, and the mean increase in plasma CPK activity at 4 hr following i.p. Cpz was not influenced by hexamethonium (Table 7). Epinephrine (1·25 mg/kg), subcutaneously produced large

TABLE 7. EFFECT OF HEXAMETHONIUM ON RECTAL TEMPERATURE AND INCREASE IN PLASMA CPK ACTIVITY FOLLOWING Cpz

Pretreatment	Plasma CPK activity ± S.D. (mU/ml)	P	Rectal temperature ± S.D. (°)	P
Saline	541 ± 295		34·4 ± 1·7	
Hexamethonium 5 mg/kg	495 ± 227	N.S.	35·1 ± 1·9	N.S.

Six rats received saline i.p. and six received hexamethonium 5 mg/kg i.p. 15 min prior to Cpz, 25 mg/kg. They were kept at 22° for 4 hr before temperature was taken and plasma obtained. All data was analysed using a paired *t*-test.

increases in plasma CPK activity with the peak at 6 hr (Table 6). The smallest dose of epinephrine which increased plasma CPK activity was 0·2 mg/kg. Plasma CPK activity was increased 6 hr after the subcutaneous injection of norepinephrine (4 mg/kg) (Table 6).

DISCUSSION

The incubation studies indicate that Cpz can produce an increased release of CPK from muscle. This could be a toxic effect of Cpz on muscle due to an interference with metabolic processes necessary for the maintenance of cell membrane integrity and permeability. An interference with the energy producing processes in skeletal muscle by Cpz has been demonstrated by Peterson *et al.*[20] The increased release of CPK *in vitro* could also be another manifestation of the many direct effects of Cpz on membrane permeability.[21] Since the enhanced release of CPK from muscle *in vitro* was dose related, it could be expected that at the site of an i.m. injection of Cpz, increased release of CPK might occur. The *in vitro* studies further demonstrate that the release of CPK from muscle is lower as the environmental temperature diminished. Similar findings were noted by Zierler[22] and Dawson[17] for the release of other enzymes from rat and chick muscle respectively. Zierler attributed this to a diminished requirement for energy at lower temperatures. This suggests that lower body temperatures following Cpz should decrease the release of CPK from striated muscle.

Neither of these conclusions is consistent with the data from the *in vivo* studies. These studies demonstrated that it was only when Cpz produced hypothermia that it increased plasma CPK activity. Blockade of the hypothermic effects of Cpz by administering it to rats kept at 31° or to cold acclimated rats prevented any rise in plasma CPK activity. Furthermore, the increased plasma CPK activity was correlated with the fall in body temperature at the 0·001 level of confidence. In adrenalectomized rats which at 8 hr after Cpz had greater hypothermia than did control rats, and in rats kept at 2° which had greater decreases in body temperature than rats kept at 22°, the increases in plasma CPK activity were significantly larger ($P < 0·001$) than in controls.

No evidence for a toxic effect of Cpz on muscle *in vivo* which manifests itself in increased plasma CPK activity was observed. I.m. and i.p. injections of Cpz in rats kept at 22° produced equivalent increases in plasma CPK activity while i.m. Cpz and saline injections to rats kept at 31° produced small, but equivalent increases in plasma CPK activity. Placing the rats at 31° 1 hr after giving them Cpz at an ambient temperature of 22° also produced no net rise in CPK activity by 4 hr after Cpz administra-

tion. Presumably increased CPK release took place at 22° but this additional CPK in plasma must have been cleared in the next 3 hr as body temperature returned to normal and the increased release stopped. Since the effect of the i.m. injection of Cpz on plasma CPK activity could be blocked by preventing temperature fall, it is unlikely that the increase in CPK activity after i.m. Cpz is due to the toxic effect of the high concentration of Cpz at the injection site.

Two lines of evidence indicate that increased secretion of adrenal corticoids after Cpz are not involved in the increased plasma CPK activity after this drug. The administration of cortisone (5 mg/kg), did not alter plasma CPK activity. Secondly, adrenalectomized rats given Cpz had increases in plasma CPK activity equivalent to those of intact rats at 4 hr, at which time they had become hypothermic to the same extent.

If direct inhibition of clearance of CPK by Cpz was the reason for the increased plasma CPK levels, Cpz should have also raised the plasma CPK activity in those instances where there was no temperature fall. This was not observed. However, if the rate of clearance of CPK from plasma diminishes at lower temperatures, then plasma CPK activity would increase as Cpz lowered the body temperature. Further study to evaluate this possibility is warranted.

The data suggests that CPK levels and body temperature following Cpz are inversely related. It is unlikely that CPK released from muscle, following Cpz, can lower body temperature. However, it is likely that the lowering of body temperature can lead to the increased levels of CPK, just as hypothermia is associated with increases in the levels of other serum or plasma enzymes.[8–11] How hypothermia produces increased levels of plasma enzymes is not clear.[8–10] We have suggested that a decreased rate of clearance at lower body temperatures should be considered. It is also possible that the physiological response(s) of the rat to counteract the hypothermia produced by Cpz (or by other means) is a cause of the increased levels of enzyme in plasma. Increased release of epinephrine and norepinephrine occurs during hypothermia. As demonstrated, norepinephrine and epinephrine in high dosages produced increased plasma CPK activity. However, hexamethonium, which should block the release of catecholamines at nerve endings by Cpz,[12] did not modify the increase in plasma CPK activity, or the hypothermia following Cpz, indicating that these catecholamines were probably not the cause of the increased plasma CPK activity. Shivering in response to hypothermia was also considered as a possible cause of the increased plasma enzyme activity since intense physical activity can cause increased plasma CPK activity.[23] However, after a 25 mg/kg dose of Cpz, we noted that the rats did not shiver, in agreement with the findings of Dundee *et al.*[19]

During severe hypothermia (11–20°) in the rat, the metabolic activities of cardiac muscle cells are disrupted and the membrane potential decreases.[24] It is possible that comparable impairment in the metabolic activities of skeletal (and possibly smooth) muscle during hypothermia can lead to increases in the rate of release of CPK from muscle. The reason why this is not observed in *in vitro* studies could be that *in vivo*, hypothermia is accompanied by significant changes in ionic permeability of the sarcolemma with large changes in the concentrations of intracellular and extracellular electrolytes, in particular, hypokalemia.[25,26] Severe hypokalemia, e.g. a serum potassium level of 1·6 mequiv./l., in man, is associated with myopathy and increased serum CPK activity.[27] However, the fact that substantial increases in CPK activity

after Cpz are observed with temperature decreases of just 1°, which are not associated with profound changes in serum potassium levels, makes it unlikely that this mechanism is responsible for the increase in plasma CPK activity. Thus, the apparent increase in permeability of muscle *in vivo* following hypothermia is, as yet, not amenable to satisfactory explanation.

Since i.m. injections of Thorazine® which produced increased plasma CPK activity in man are not associated with lowered body temperature, hypothermia is not the cause for the increased plasma CPK levels in man. It is possible that the ability of Cpz to release CPK from muscle as demonstrated *in vitro*, together with an inability to clear the enzyme from plasma rapidly is the cause of the plasma CPK increase in man. Although we found no greater increase in plasma CPK activity by Thorazine® than by Cpz in the rat, Warnock and Ellman noted that the vehicle used to dissolve Cpz in Thorazine® could produce a 4-fold increase in CPK activity in the rabbit.[3] It is possible that in man the vehicle used in Thorazine® contributes to the increase in plasma CPK activity after Thorazine®.

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REFERENCES

1. H. MELTZER, *Arch. Gen. Psychiat.* **21**, 102 (1969).
2. H. MELTZER, L. ELKUN and R. MOLINE, *Arch. Gen. Psychiat.* **21**, 731 (1969).
3. D. G. WARNOCK and G. L. ELLMAN, *Science* **164**, 724 (1969).
4. H. Y. MELTZER, L. GRINSPOON and R. I. SHADER, *Comp. Psych.* **11**, 552 (1970).
5. H. Y. MELTZER, S. MROZAK and M. BOYER, *Am. J. med. Sci.* **259**, 42 (1970).
6. P. J. GIELEN, *Ann. biol. Clin.* **24**, 451 (1966).
7. E. BLAIR, R. HOOK, H. TOLLEY and G. E. BUNCE, *Science* **133**, 105 (1961).
8. B. HIGHAM and P. D. ALTLAND, *Proc. Soc. exp. Biol. Med.* **109**, 523 (1962).
9. P. D. ALTLAND, B. HIGHMAN and M. PARKER, *Proc. Soc. exp. Biol.* **123**, 853 (1966).
10. H. Y. MELTZER, *Fedn Proc.* **29**, 747 (Abst.) (1970).
11. S. COURVOISER, J. FOURML and T. DUCROT, *Archs int. Pharmacodyn.* **92**, 305 (1953).
12. G. E. JOHNSON, *Acta physiol. scand.* **60**, 181 (1964).
13. B. HIGHAM, H. M. MALING and E. C. THOMPSON, *Am. J. Physiol.* **196**, 436 (1959).
14. A. N. BHATTACHARYA and B. H. MARKS, *J. Pharmac. exp. Ther.* **165**, 108 (1969).
15. F. SCHAPIRA, *C. r. Soc. Biol.* **148**, 1997 (1954).
16. W. PEARL, T. BALAZS and D. A. BUYSKE, *Life Sci.* **5**, 67 (1966).
17. D. M. DAWSON, *Biochim. biophys. Acta* **113**, 144 (1966).
18. S. B. ROSALKI, *J. Lab. clin. Med.* **69**, 696 (1967).
19. J. W. DUNDEE, P. R. MESHAM and W. E. B. SCOTT, *Anaesthesiol.* **9**, 296 (1954).
20. R. D. PETERSON, C. H. BEATTY, R. M. BOCEK and H. H. DIXON, *Bull. Tulane U. Med.* **25**, 21 (1966).
21. P. S. GUTH and M. A. SPIRTES, in *International Review Neurobiology*, Vol. 7 (Eds. C. C. PFEIFFER and J. R. SMYTHIES), Academic Press, New York (1964).
22. K. L. ZIERLER, *Am. J. Physiol.* **185**, 1 (1956).
23. P. D. GRIFFITHS, *Clin. chim. Acta* **13**, 413 (1966).
24. M. L. ZIMNY and S. TAYLOR, *Am. J. Physiol.* **208**, 1247 (1965).
25. A. VAN HARREVELD and S. OCHS, *Am. J. Physiol.* **180** (1956).
26. W. R. BEAVERS and J. T. RODGERS, JR., *Am. J. Physiol.* **196**, 706 (1959).
27. G. VAN HORN, J. B. DRORI and F. S. SCHWARTZ, *Archs Neurol.* **22**, 335 (1970).