EFFECTS OF SEASONAL VARIATIONS, ACUTE HYPOTHERMIA AND PHYSOSTIGMINE ON CARDIAC ACETYLCHOLINE, TISSUE GLYCOGEN AND BLOOD SUGAR CONCENTRATIONS IN FROGS

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Summary: The effects of seasonal variations and the effects of acute hypothermia (8.0°) and/or physostigmine (PHY) in different seasons have been studied on the tissue glycogen, cardiac acetylcholine (ACh) and blood sugar contents in frogs. Seasonal variations had no significant effect on cardiac cholinergic activity. However, cardiac ACh concentration was significantly reduced by hypothermia in all seasons. The extents of increase in cardiac ACh in PHY pretreated hypothermic frogs indicate that hypothermia depresses cardiac cholinergic activity much more in summers than in rainy and winter seasons.

The tissue glycogen contents and blood sugar concentrations were significantly lower in winter season than those in summer and rainy seasons. Hypothermia produced marked tissue glycogenolysis and hyperglycaemia during summer and rainy seasons and not during winters. In general PHY had no effect on tissue glycogen contents in any season, but it produced hyperglycaemia during rainy and winter seasons. PHY pretreatment increased cardiac, hepatic and muscle glycogen contents and produced hyperglycaemia in hypothermic winter frogs, and it increased ventricular and muscle glycogen contents during summer and hepatic glycogen during rainy seasons, there being no significant effect on blood sugar.

The results indicate that even though frog is a poikilothermic animal, reacts adversely to acute cooling particularly in summer months. In addition, the cholinergic system functions normally in all seasons. An anti-cholinesterase agent can prevent tissue glycogenolysis produced by hypothermia in all seasons.

Key words: seasonal variations, hypothermia, Physostigmine, Acetylcholine, glycogen, Frog

INTRODUCTION

The effect of body temperature on the physiological and biochemical functions have been a matter of interest for the last several decades. The researchers have mainly concentrated on either the effect of hypothermia in homeothermic mammals or the process of hibernation in hibernating mammals. However, not much interest has been evinced in poikilothermic animals. The interest in the poikilothermic animals. is specially because of the fact that, there being practically no system of thermoregulation, the physiological and biochemical functions of the body are geared to function optimally at a wide range of atmospheric as well as body temperatures. The adaptability to temperature in these species, therefore, is expected to be much better than that of homeothermic mammals. There is no doubt that during winter season when the atmoshperic temperature is low, the functional activity of the body in the poikilothermic animals like frogs is sluggish. It has been shown that the ciliary movements of the oesophagus (4) and the vascular responses of frogs (7) are sluggish during winter months. However, if the frogs are subjected to acute hypothermia, tissue glycogenolysis and hyperglycemia have been observed (14). Physostigmine, which increases cardiac acetylcholine (Ach) contents without having any effect on tissue glycogen contents in normothermic frogs (5) have been shown to prevent tissue glycogen depletion produced by acute hypothermia (14). It was, therefore, considered worthwhile to study the effect of seasonal variations and the effects of acute hypothermia and/or physostigmine in different seasons on the tissue glycogen, cardiac ACh and blood sugar contents, with the idea of investigating the differences, if any, between acute cooling and cooling due to change in season.

MATERIALS AND METHODS

Experiments were conducted on frogs (*Rana tigrina*) of either sex weighing between 150-350 g. The studies were conducted during the months of summer (May-June), rainy (July-October) and winter (December-February) seasons. The mean minimum and maximum environmental temperatures during these seasons had been 28 and 41°C, 25 and 33°C and 8 & 23°C, respectively.

Hypothermic state was produced in frogs by keeping them in a refrigerated chamber with a temperature of 8°C for a period of 2 hr. Cardiac ACh, tissue glycogen and blood sugar concentrations were estimated. The protocols were as follows :

Group (a) the effect of different seasons on the normal values;

Group (b) the effect of physostigmine in different seasons;

Group (c) the effect of hypothermia in different seasons and

Group (d) the effect of hypothermia in physostigmine pretreated frogs in different seasons.

Physostigmine salicylate (1 *mg/kg*) (PHY) was administered through the ventral lymph sac. In the case of group (d) experiments, PHY was administered 30 min before exposing the frogs to a refrigerated chamber. Blood for sugar estimations was collected twice, once in the beginning of the. experiment and again at the termination of the experiment. Tissues for biochemical estimations were collected one hr after drug administration for group (b) and two hr after hypothermia for groups (c) and (d). The experiments were conducted during the day time when the ambient temperatures had been nearer the maximum. The following estimations were made:—

(i) Cardiac ACh of both the auricles and the whole ventricle was extracted by the method of Nachmansohn as described by Anand (1) and assayed on eserinised frog rectus abdominis muscle by the method of Richter and Crossland (13).

(ii) Tissue glycogen contents of the apex of the ventricle, middle lobe of the liver and upper part of the gastrocnemius muscle were estimated by the phenol and sulphuric acid method as described by Montgomery (10).

(iii) Blood sugar was estimated by the method of Folin and Wu (6).

The results have been expressed as mean ± S.E., and analysed by the Student's 't' test for significance.

RESULTS

Effects of seasonal variations, hypothermia and/or physostigmine (PHY) on cardiac ACh, tissue glycogen and blood sugar concentrations : The results are summarised in Table I and II.

Seasonal variations: Variation in tne seasons had no significant effect on the auricular and ventricular ACh contents. There was no significant difference in the tissue glycogen contents during the summer and rainy seasons, but during winter season, the ventricular, hepatic as well as muscle glycogen contents were significantly lower than those of summer and rainy seasons. Maximum reduction, however, was in the hepatic glycogen content. The blood sugar concentrations varied significantly in different seasons. The normal blood sugar was highest during rainy season and minimum during winter season, while during summer it was slightly but significantly higher than that of the winter (Tables I and II, Fig. 1).

Hypothermia: Hypothermia produced a marked reduction in the auricular and ventricular ACh contents. There was no significant difference in the extent of reduction of cardiac ACh in different seasons (Table I and Fig. 1). Hypothermia produced moderate to marked glycogenolysis in all the tissues during summer and rainy seasons. The glycogenolysis was maximum in the liver. During the winter months, however, hypothermia did not produce any significant change in the tissue glycogen contents. During summer and rainy seasons hypothermia produced marked hyperglycaemia, the effect being maximum during the rainy season. During the winter, the increase in blood sugar produced by hypothermia was insignificant. A comparison of the tissue glycogen contents in hypothermic frog in different seasons shows that there was no significant effect of seasonal variations in the absolute tissue glycogen contents in hypothermic frogs (Table II). However, in the hypothermic frogs the blood sugar concentration was highest during rainy season and lowest during winter months (Fig. 1).

Physostigmine: PHY increased the auricular ACh content in rainy and winter seasons and ventricular ACh contents in summer and winter seasons. Seasonal variations had no significant effect on auricular and ventricular ACh contents in PHY pretreated frogs, except for the fact that during winters the ventricular ACh content was highest (Table I). PHY did not produce any significant change in the tissue glycogen contents in different seasons, *except that it produced a significant decrease in the ventricular glycogen content during winter* season and a *significant increase in the skeletal muscle glycogen content during summer. PHY*, however, had a very widely variable effect on the blood sugar; it had no significant effect during summer, it increased the blood sugar by about 50% during rainy season and during winter the increase was by about 200% (Fig. 2). In terms of absolute values, the blood sugar con-

Table I: Effects of seasonal variations, hypothermia and physostigmine (PHY) on acetylcholine (ACh) contents of frog heart.

Season	Groups	Mean cardiac ACh content (μg/g ±S. E.)	
		Auricle	Ventricle
Summer	Control 1	2.2±0.3 (20)	0.7±0.1 (20)
	PHY	2.4±0.1 (10)	0.9±0.1 $P<0.05$ (10)
	Hypothermia	0.9±0.1 $P<0.001$ (13)	0.5±0.1 $P<0.05$ (13)
	PHY+hypothermia	1.2±0.3 $P_1<0.01$ (10)	0.6±0.1 $P_1<0.025$ (10)
Rainy	Control 2	1.6±0.3 (5)	0.7±0.1 (5)
	PHY	2.4±0.2 $P<0.05$ (10)	0.8±0.1 (10)
	Hypothermia	0.6±0.1 $P<0.01$ (9)	0.3±0.1 $P<0.001$ (9)
	PHY+hypothermia	1.4±0.2 $P_1<0.001$ $P_2<0.001$ (10)	1.0±0.1 $P_2<0.001$ (10)
Winter	Control 3	1.9±0.2 (6)	0.8±0.1 (6)
	PHY	2.5±0.2 $P<0.05$ (5)	1.5±0.1 $P<0.001$ (5)
	Hypothermia	0.5±0.1 $P<0.001$ (5)	0.3+0.1 $P<0.001$ (5)
	PHY+hypothermia	1.1±0.4 $P_1<0.01$ (10)	0.6±0.03 $P_1<0.001$ $P_2<0.01$ (10)

Numerals in parentheses indicate 'n'

P, P_1 and P_2 in relation to respective control, PHY and hypothermia groups respectively.

TABLE II: Effects of seasonal variations, hypothermia and physostigmine (PHY) on tissue glycogen contents and blood sugar level in frogs.

Season	*Groups*	*Mean tissue glycogen content (mg/g ±S.E.)*			*Mean blood sugar level (mg/100 ml± S.E.)*
		Ventricle	*Liver*	*Sk. Muscle*	
Summer	Control 1	9.9±0.7 (20)	83.5±4.9 (20)	8.1±0.6 (20)	26.7±2.4 (20)
	PHY	11.8±1.6 (10)	71.6±5.9 (10)	11.1±0.7 P<0 .01 (10)	29.4±3.1 (10)
	Hypothermia	6.8±1.0 P<0.025 (16)	31.8±5.8 P<0.001 (15)	5.0±0.5 P<0.001 (17)	44.8±2.6 P<0.001 (17)
	PHY±hypothermia	10.1±0.6 P_2<0.01 (10)	26.4±2.7 P_1<0.001 (10)	6.8±0.7 P_1<0.001 P_2<0.05 (10)	41.3±3.1 P_1<0.001 (10)
Rainy	Control 2	11.2±0.6 (5)	96.1±6.8 (5)	8.9±0.9 (5)	42.9±2.8 P_3<0.001 (5)
	PHY	9.8±0.7 (10)	102.7±8.3 (10)	6.9±0.5 (10)	63.1±3.8 P<0.001 (10)
	Hypothermia	7.8±0.7 P<0.001 (5)	39.3±3.6 P<0.001 (6)	6.0±0.8 P<0.05 (5)	87.9±9.7 P< 0.001 (5)
	PHY+Hypothermia	9.4±0.4 (7)	67.2±8.3 P_1<0.01 P_2<0.01 (7)	6.0±0.3 (8)	98.2±8.6 P_1<0.001 (5)
Winter	Control 3	6.7±0.2 P_3<0.001 P_4<0.001 (7)	36.2±3.1 P_3<0.001 P_4<0.001 (5)	5.8±0.4 P_3<0.001 P_4<0.001 (8)	20.9±1.0 P_3<0.01 P_4<0.001 (11)
	PHY	4.4±0.7 P<0.01 (5)	34.5±4.1 (5)	4.0±0.4 (5)	63.6±8.9 P<0.001 (5)
	Hypothermia	5.8±0.6 (5)	30.0±4.4 (5)	5.2±0.5 (5)	28.9±7.0 (6)
	PHY+hypothermia	9.9±0.7 P_1<0.001 P_2<0.001 (10)	57.7±3.6 P_1<0.001 P_2<0.001 (10)	7.3±0.4 P_1<0.001 P_2<0.01 (10)	54.5±2.9 P_2<0.001 (10)

Numerals in parentheses indicate 'n' P, P1 and P2 in relation to respective control, PHY and hypothermia groups. P_3 and P_4 in relation to control-1 and Control—2, respectively.

centration in PHY pretreated frogs was nearly the same in rainy and winter seasons, which were about twice that of summer season (Table II).

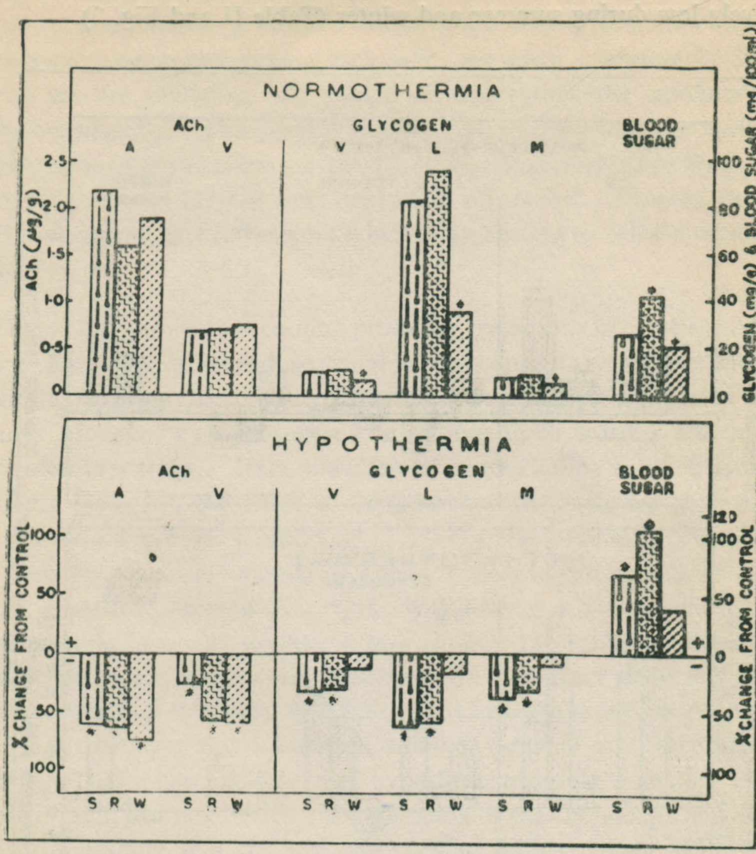

Fig. 1: Acetylcholine (ACh) concentrations of auricle (A) and ventricle (V); glycogen content of ventricle (V), liver (L) and skeletal muscle (M) and blood sugar concentrations in normothermic and hypothermic frogs during summer (S), rainy (R) and winter (W) seasons.

Bars in normothermia panel indicate absolute values, and those in hypothermia panel indicate values as percentage change (+or —) in respect of controls (normothermia).

Physostigmine in hypothermic frogs: PHY increased the cardiac ACh contents in hypothermic frogs in all the seasons. However, the increase in the auricular and ventricular ACh contents was relatively low (+36% & 23%) during summers as compared to more than 100% increase during the rainy and winter seasons (Table I). PHY pretreatment significantly increased the hepatic glycogen content during winter and rainy seasons and the ventricular and skeletal muscle glycogen contents during summer and winter in hypothermic frogs. During summer and rainy seasons PHY had no significant effect on blood sugar, but during winter season it produced marked increase in the blood sugar. A comparison of the absolute values of glycogen content in PHY pretreated hypothermic frogs shows that seasonal variation had no effect in the ventricular and skeletal muscle glycogen contents, but the hepatic glycogen contents during rainy and winter seasons were more than twice than that of the summer. The absolute blood sugar concentration in PHY pretreated hypothermic frogs was highest in rainy season and relatively low during summer and winter (Table II and Fig. 2).

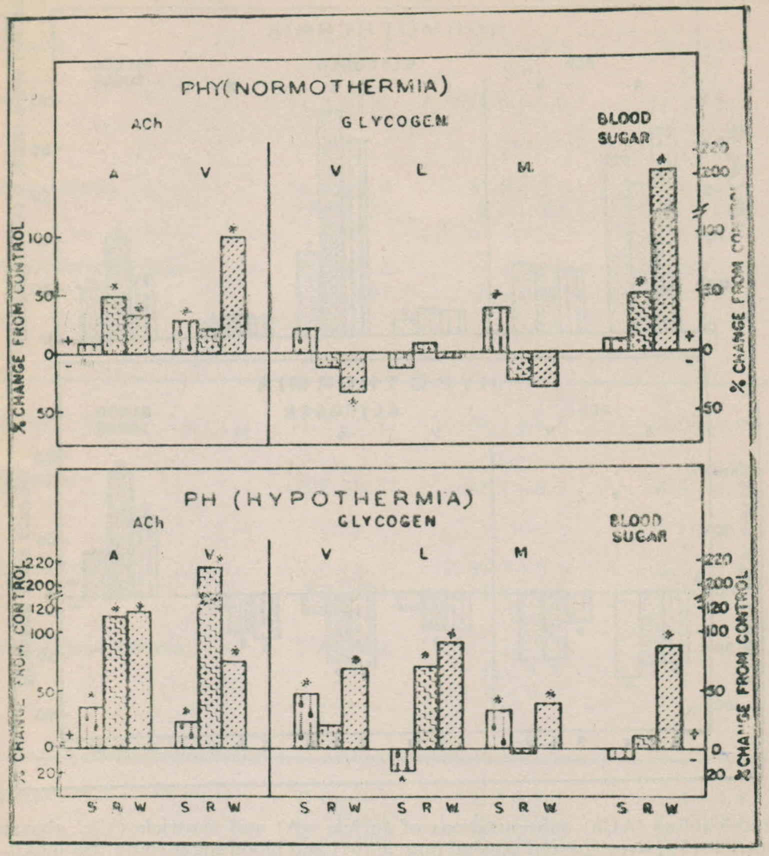

Fig. 2: Effects of physostigmine (PHY) on acetylcholine (ACh) concentrations of auricle (A) and ventricle (V); glycogen contents of ventricle (V), liver (L) and skeletal muscle (M) and blood sugar concentrations in normothermic and hypothermic frogs during summer (S), rainy (R) and winter (W) seasons.

Bars indicate values as percentage change (+ or —) in respect of corresponding controls (normothermia/hypothermia).

DISCUSSION

Poikilothermic animal *Rana temporaria* has been reported to have reduced total glycogen and liver glycogen contents from September to February (2,9,15) alongwith rise in blood sugar before hibernation and hypoglycaemia during hibernation and early summer (15). The present studies with *Rana tigrina* confirm the above findings. The marked reduction in the hepatic glycogen content during winter shows that during the hibernating period the body is mostly dependent on and also using up hepatic glycogen stores.

Seasonal variations had no significant effect on cardiac ACh concentrations. The increase in the cardiac ACh concentration following an anticholinesterase agent (anti-ChE) indicates indirectly the cholinergic activity, as it has been shown that PHY increases cardiac ACh content in control and atropine pretreated dogs but not after a ganglion blocking agent (12). Therefore, a similar increase in the cardiac ACh following PHY treatment in different seasons indicates that in all seasons including the period of hibernation the cholinergic activity is maintained nearly consistently. The present studies while confirming the earlier report that anti-ChE agent has no significant effect on the tissue glycogen content of frogs (5) also show that the effect of PHY by and large is not dependent on season. Howevr, during rainy and winter season PHY produces hyperglycaemia which may be due to release of adrenaline from the adrenal medulla.

An earlier study has shown that cooling produces reduction in cardiac ACh concentrations (14). The present study shows that seasonal variation has no significant effect in the extent of reduction in cardiac ACh produced by hypothermia. It is interesting to note that the cardiac ACh concentrations produced by hypothermia in all the three seasons are much lower than the normal values of winter frogs. Thus, the effect of acute cooling is much different than that of seasonal cooling. However, the effect of hypothermia on the tissue glycogen content and blood sugar concentrations are quite different. Hypothermia produced generalised tissue glycogenolysis and hyperglycaemia in summer and rainy seasons while it had no significant effect during winter. It, therefore, appears that when the atmospheric temperature is high, frogs also react to hypothermia in a way similar to that of dogs (3), while during winter frogs are better adapted to cold environment and, therefore, do not react adversely to cooling even though the diurnal variation of temperature is high. The increase in cardiac ACh in hypothermic frogs following PHY treatment was smaller in summer while it was very high during rainy and winter seasons. This might indicate that hypothermia during summer, when the atmospheric temperature is high, produces much more damping of the cholinergic activity than in other seasons. It is worth nothing that the absolute cardiac ACh contents in PHY treated hypothermic frogs in all the seasons were mostly not only lower than those of PHY treated normothermic frogs but also those of control frogs. These findings clearly indicate the significant diminution in the cholinergic activity produced by hypothermia in all seasons.

In earlier studies which were conducted during summer and rainy seasons without analysing the effect of seasonal variation it was reported that PHY pretreatment prevents tissue glycogen depletion without affecting hyperglycaemia produced by hypothermia (14). The present study shows that the effect of PHY on the tissue glycogen and blood sugar in hypothermic frogs is partially dependent on the seasons. During winter, PHY significantly increased glycogen content in all the tissues and also produced hyperglycaemia in hypothermic frogs. The effects in rainy and summer seasons were not so consistent.

Several researches (8,11,16) have shown adaptation or "acclimatization' phenomenon in poikilothermic animals. The physical, physiological and biochemical activities are highest during the phase of high environmental temperature and lowest during the phase of winter hibernation. It was interesting to note that even during winters the cholinergic neuronal activity was not depressed. The present study also indicates that, though frogs are expected to have better 'adaptation' mechanism to variations in ambient temperature, acute hypothermia acts as a stress producing tissue glycogenolysis and hyperglycaemia when ambient temperature is high but not so during winters. But the cholinergic activity is consistently depressed by acute cooling irrespective of ambient temperature. Prevention of tissue glycogenolysis by anti-ChE agent in hypothermic frogs is confirmed. However, the effect is more consistent during winters than in summer or rainy season. The reason of this difference may be due to the fact that when the atmospheric temerature is high, acute cooling also acts as a stress.

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