

cyclone track just before crossing the coast is important for accurately predicting the landfall. Another powerful tool used is remote sensing to map the regional geomorphology of the terrain. The database generated can be updated regularly whenever a new cyclone develops and crosses the coast.

The study has revealed that accurate prediction can be made on the landfall of cyclone when the eye of the cyclone is within the range of 100 km from the coast. This is one step forward towards better disaster monitoring and management, even though the time required for taking disaster mitigation measures is less.

1. IMD Atlas, Tracks of storms and depression crossing Bay of Bengal - 1877-1995.
2. Jayanti, N. and Sen Sarma, A. K., *Mausam*, 1988, 38, 193-196.
3. *Coastal Meteorology*, National Research Council, National Academy Press, Washington DC, 1992.

4. Abbs, J. D., *Mon. Weather Rev.*, 1986, 114, 881.
5. Abe, S. and Yashida, T., *J. Meteorol. Soc. Japan*, 1982, 60, 1074-1084.
6. Atlas, D., Schu Hseien Chow and William, D., *Mon. Weather Rev.*, 1983, 111, 2, 24.
7. Mahru, Y. and Seegal, M. C., *Mon. Weather Rev.*, 1985, 113, 170.
8. Chang, S. W., *Mon. Weather Rev.*, 1982, 110, 1255-1270.
9. Mams, A., Tuley, E. R. and Yoshro Kurihara, *Mon. Weather Rev.*, 1987, 115, 130.
10. Jones Rober, W. A., *Mon. Weather Rev.*, 1987, 115, 2279.
11. Chang T'ytai and Wetzel, P. J., *Mon. Weather Rev.*, 1991, 112, 1369.

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## *In vitro* effects of adrenergic agonists and antagonists on tissue respiration in *Rana limnocharis* and *Rana cyanophlyctis*

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*In vitro* effects of  $\alpha$ 1- and  $\beta$ 1-adrenergic agonists, phenylephrine (PHE) and isoproterenol (ISO), on the rate of tissue respiration of *Rana limnocharis* and *Rana cyanophlyctis* were studied in the presence and absence of  $\alpha$ 1- and  $\beta$ 1-adrenergic antagonists, prazosin (PRAZ) and propranolol (PROP), during the month of April (summer/rainy season). PHE and ISO, when administered separately, induced significant increase in the rate of liver and muscle tissue respiration. ISO and PHE, when administered together, potentiated the calorogenic action of each other. PHE-induced increase in the respiratory rate was blocked significantly by PRAZ only in muscles of both the species. PROP blocked ISO-stimulated respiratory rate of liver tissue of *Rana limnocharis* and of skeletal muscle of *Rana cyanophlyctis*. However, PRAZ and PROP when administered together, completely blocked the ISO- or PHE-induced increase in the rate of tissue respiration in both the species. These findings seem to confirm that both  $\alpha$ 1- and  $\beta$ 1-adrenergic receptors are actively involved in the adrenergic stimulation of the metabolic rate of amphibian tissues. The degree of involvement of the adrenergic receptors in calorogenesis seems to vary with the tissues and the species.

CATECHOLAMINE hormones, norepinephrine (NE) and epinephrine (EP); play a critical role in the regulation of the oxidative metabolism and calorogenesis in all groups

of vertebrates except birds<sup>1-7</sup>. NE and EP have been reported to regulate the energy metabolism of mammals at low temperatures and during emergency<sup>1,2</sup>. Similarly, due to their temperature-independent and rapid calorogenic action, these hormones act as emergency hormones for the regulation of the metabolic rate in poikilothermic vertebrates as well<sup>5-11</sup>. NE and EP have been reported to stimulate respiration of brown adipose tissue in mammals, with the involvement of  $\alpha$ 1- as well as  $\beta$ 1-adrenergic receptors<sup>12,13</sup>. Our recent studies indicate that as in mammals, catecholamines produce their calorogenic effects in amphibians as well through  $\alpha$ 1- and  $\beta$ 1-adrenergic receptors<sup>9</sup>. However experiments, using specific  $\alpha$ 1- and  $\beta$ 1-adrenergic agonists and antagonists, were needed to support our initial studies<sup>9-11</sup>. Therefore, we investigated *in vitro* effects of isoproterenol (ISO; a  $\beta$ 1-adrenergic agonist) and phenylephrine (PHE; an  $\alpha$ 1-adrenergic agonist) in the absence and presence of prazosin (PRAZ; specific blocker of  $\alpha$ 1-adrenergic receptors) and propranolol (PROP; specific blocker of  $\beta$ 1-adrenergic receptors) on the rate of tissue (liver and skeletal muscle) respiration in *Rana limnocharis* (a hibernating species) and *Rana cyanophlyctis* (a non-hibernating species).

All *in vitro* experiments were conducted on liver and muscle tissues collected from adult male Indian streaked frog, *Rana limnocharis* (a hibernating species; body weight 8-10 g; snout to vent length, 31-35 mm) and Indian skipper frog, *Rana cyanophlyctis* (a nonhibernating species; body weight, 10-12 g; snout to vent length, 35-40 mm). These two species of frog are commonly found in and around Shillong (lat. 25°30'N; long. 91°52'E; alt. 1450 m above sea level; minimum temperature, 2°-4°C; maximum temperature, 25°-28°C). Frogs were collected locally during the month of April (maximum temperature 23°C; minimum temperature

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11°C). Four frogs of each species were decapitated immediately after collection and tissues (liver and skeletal muscle) were rapidly removed, rinsed in ice-cold frog Ringer's solution and stored in a deep freezer at -15°C to -18°C for *in vitro* experiments. For treating with adrenergic agonists and antagonists, tissues were blotted, weighed and homogenized in ice-cold frog Ringer-phosphate buffer solution (9:1) (pH 7.4) with the help of a glass homogenizer. One ml of the tissue homogenate (16–20 mg tissue/ml), 3.9 ml of the Ringer-phosphate buffer and 0.1 ml of solution containing adrenergic agonists and/or antagonists were used for measuring the rate of tissue respiration. The homogenates were treated with phenylephrine (1 µM) and isoproterenol (1 µM) separately or in combination in the presence and absence of prazosin (1 µM) and/or propranolol (1 µM). In the combination treatments, the homogenates were first incubated with the adrenergic antagonists PRAZ and PROP at 4°C for 1 h and then with the agonists PHE and ISO in the incubation chamber of the oxygen electrode for 20 min before recording the rate of tissue respiration.

The rate of tissue oxygen consumption was measured with an oxygen electrode (Digital Oxygen System Model 10, Rank Brothers Ltd., UK). The polarizing voltage was kept at 0.6 volt and the homogenizing medium was used as the polarizing medium. Since the preferred body temperature of most amphibians ranges between 25–28°C, temperature of the incubation chamber of the electrode was maintained at 25°C using a thermostat-controlled water circulator. The homogenates were incubated in the incubation chamber of the oxygen electrode for 20 min before recording the readings. Readings for each tissue were taken for 30 min in the linear range of oxygen consumption. The rate of tissue respiration was calculated and expressed as µl O<sub>2</sub>/mg wet tissue/hour<sup>9</sup>. The data were tabulated and analysed with the help of

Student's *t* test. A probability of less than 5% was considered to be significant<sup>14</sup>.

The data is presented in Table 1. PHE as well as ISO when added separately to the homogenates, showed a significant increase in the respiratory rate of the tissues of *Rana limnocharis* and *Rana cyanophlyctis*. On the other hand when ISO and PHE were added together to the homogenates, they potentiated the stimulatory effects of each other in the tissues of both the species. PRAZ significantly blocked the PHE-induced increase in the respiratory rate of only muscle tissues of both the species. PROP significantly reduced the stimulatory effect of ISO in the liver of *Rana limnocharis* and in the muscle tissue of *Rana cyanophlyctis*. When the tissue homogenates were pre-incubated with a combination of PRAZ and PROP, a significant block in the stimulatory effects of ISO as well as PHE was observed on the respiratory rate of liver and muscle tissues of *Rana limnocharis* as well as *Rana cyanophlyctis*. The blocking effect of the combined treatment with PRAZ and PROP was more prominent compared to their individual inhibitory effect.

Findings of the present study suggest that both α1- and β1-adrenergic receptors are involved in the calorigenic action of adrenergic agonists in *Rana limnocharis* and *Rana cyanophlyctis* irrespective of their hibernating and nonhibernating habits. Though both ISO and PHE increased the respiratory rate of liver and muscle tissues, they were more effective in stimulating muscle respiration as compared to that of liver of both the species (Table 1). Further, the liver of *Rana cyanophlyctis* seems to be more responsive to the adrenergic agonists as compared to that of *Rana limnocharis*. Further, compared to the calorigenic effect of each agonist separately, the combined treatment was more effective in stimulating tissue respiration. It, thus, seems that α1- as well as β1-adrenergic receptors are capable of stimulating tissue respiration in frogs, while simultaneous stimulation of

Table 1. *In vitro* effects of α1- and β1-adrenergic agonists and antagonists on the rate of tissue respiration of *Rana limnocharis* and *Rana cyanophlyctis*

Treatments	Rate of oxygen consumption (µl O <sub>2</sub> /mg wet tissue/h)			
	<i>Rana limnocharis</i>		<i>Rana cyanophlyctis</i>	
	Liver	Muscle	Liver	Muscle
Saline (control)	0.88 ± 0.04 <sup>b</sup>	0.54 ± 0.08	0.78 ± 0.03	0.41 ± 0.07
Phenylephrine (PHE)	1.00 ± 0.03 <sup>a</sup>	0.83 ± 0.03 <sup>a</sup>	0.98 ± 0.07 <sup>a</sup>	0.62 ± 0.01 <sup>a</sup>
Isoproterenol (ISO)	1.02 ± 0.01 <sup>a</sup>	0.84 ± 0.09 <sup>a</sup>	1.03 ± 0.09 <sup>a</sup>	0.64 ± 0.03 <sup>a</sup>
PHE + ISO	1.30 ± 0.07 <sup>b</sup>	1.06 ± 0.03 <sup>a</sup>	1.31 ± 0.12 <sup>b</sup>	0.82 ± 0.09 <sup>a</sup>
PHE + Prazosin (PRAZ)	0.97 ± 0.03	0.67 ± 0.02 <sup>c</sup>	0.84 ± 0.05	0.49 ± 0.01 <sup>f</sup>
ISO + Propranolol (PROP)	0.94 ± 0.02 <sup>c</sup>	0.63 ± 0.05	0.82 ± 0.07	0.42 ± 0.03 <sup>f</sup>
PHE + PRAZ + PROP	0.0 ± 0.07 <sup>ac</sup>	0.56 ± 0.03 <sup>f</sup>	0.58 ± 0.08 <sup>f</sup>	0.35 ± 0.03 <sup>f</sup>
ISO + PRAZ + PROP	0.57 ± 0.09 <sup>ac</sup>	0.44 ± 0.07 <sup>f</sup>	0.55 ± 0.02 <sup>c</sup>	0.32 ± 0.07 <sup>f</sup>

\*Mean ± Standard error; N = 4.

<sup>a,b,c</sup> Differ from the saline treated control group; *P* < 0.05, 0.01 and 0.001, respectively.

<sup>d,e,f</sup> Differ from respective agonist (ISO or PHE) treated group; *P* < 0.05, 0.01 and 0.001, respectively.

both the isoreceptors might be leading to higher metabolic rate. This suggestion is further supported by the findings that  $\alpha$ 1- and  $\beta$ 1-adrenergic antagonists (PRAZ and PROP), when administered together, were more effective in blocking the stimulatory effects of the agonists compared to their individual inhibitory effects. These findings suggest that, unlike in mammals<sup>12,13</sup>, the  $\alpha$ 1- and  $\beta$ 1-adrenergic receptors present in amphibian tissues exhibit certain degree of cross-reactivity for different agonists. In mammals,  $\beta$ 1-adrenergic receptors stimulate tissue respiration using cAMP as a second messenger, while  $\alpha$ 1-adrenergic receptors mediate the calorogenic action of adrenergic agonists via  $\text{Ca}^{++}$  and phosphatidyl inositol metabolites ( $\text{IP}_3$  and DAG)<sup>12,13</sup>. A similar mechanism might also be operational during adrenergic agonists-induced stimulation of tissue respiration in amphibians<sup>11</sup>.

On the basis of the present findings, it can be suggested that the adrenergic agonists involve both  $\alpha$ 1- and  $\beta$ 1-adrenergic receptors for stimulating tissue respiration in amphibian tissues. However, the degree of stimulation of tissue respiration by the agonists seems to be tissue- and species-dependent. Whether the observed differences in the calorogenic response of the tissues to adrenergic agonists is a kind of adaptation for hibernating and nonhibernating species remains to be explored.

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1. Himms-Hagen, J., in *Handbook of Physiology* (ed. Geiger, S. R.), Am. Physiol. Soc., Washington DC, 1975, vol. VI, sect. 7, pp. 637-665.

2. Landesberg, L. and Young, J. B., in *William's Textbook of Endocrinology* (eds Wilson, J. D. and Foster, D. F.), W. B. Saunders, London, 1985, pp. 891-965.
3. Gupta, B. B. P. and Thapliyal, J. P., *J. Endocrinol.*, 1983, **99**, 211-216.
4. Palonkangas, R. and Hissa, R., *Comp. Biochem. Physiol.*, 1971, **A38**, 743-750.
5. Gupta, B. B. P. and Mahanta, A., *Indian J. Exp. Biol.*, 1997, **35**, 244-249.
6. Gupta, B. B. P. and Chakrabarty, P., *Indian J. Exp. Biol.*, 1990, **28**, 23-26.
7. Gupta, B. B. P. and Deka-Borah, H., *Indian J. Exp. Biol.*, 1995, **33**, 604-607.
8. Banerjee, S. and Joshi, S. C., *Indian J. Exp. Biol.*, 1981, **19**, 982-983.
9. Gupta, B. B. P. and Mahanta, A., *Indian J. Exp. Biol.*, 1997, **35**, 1336-1340.
10. Thapliyal, J. P. and Gupta, B. B. P., in *Recent Trends in Life Sciences* (eds Gopalakrishna, A. et al.), Manu Publication, Kanpur, 1983, pp. 260-275.
11. Gupta, B. B. P. and Thapliyal, J. P., *Zool. Sci.*, 1991, **8**, 625-638.
12. Mohell, N., Nedergaard, J. and Cannon, B., *Euro. J. Pharmacol.*, 1983, **93**, 183-193.
13. Mohell, N., Wallace, M. and Fain, J. N., *Molecular Pharmacol.*, 1983, **25**, 64-69.
14. Snedecore, G. W., *Statistical Methods*, Pacific Pvt. Ltd., Bombay, 1961.

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