formulation of 5 μg, 0.4 ml (10 μg) of stock solution, 9 ml of 1M sodium hydroxide were added and heated for 1h in water bath at 95°. The volume was made upto 25 ml with distilled water to get a concentration of 0.6 μg/ml and was reanalyzed as mentioned earlier. The recovery was close to 100 % indicating the reproducibility and accuracy of the method.

The fluorescence intensity has linear relationship in the concentration range of 0.2-1 μg/ml. The quantum efficiency (Ø) was calculated for the present method using reported method and was found to be 0.9767 using quinine sulphate as reference[7]. Stability study proved that the developed fluorophore was stable upto 2 h at room temperature (28±1°). After that fluorescence intensity diminished gradually. The LOD and LOQ were found to be 0.04 and 0.2 μg/ml, respectively (Table 2). Thus the developed method was specific, accurate, simple, precise and reproducible, implying its use in the routine analysis of cefdinir in formulation.

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Involvement of Potassium Channels in the Release of Various Hormones

ARCHANA N. PARANJAPE, D. D. SANTANI, R. K. GOYAL, ANITA A. MEHTA*

Department of Pharmacology, L. M. College of Pharmacy, Navrangpura, Ahmedabad-380009.

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The present investigation was taken up to study the effect of long term treatment (30 days) of various potassium channel openers and blockers on serum insulin, glucose, T_3, TSH and plasma cortisol in rats. Treatment with cromakalim for 30 days produced a significant decrease in serum insulin levels in rats. However, treatment with pinacidil and glibenclamide produced a significant increase in serum insulin levels. KRN 2391 did not produce any effect on serum insulin levels. Glucose levels were decreased significantly only with glibenclamide and no significant alteration in serum glucose levels was observed with any of the potassium channel openers. Serum T_3 levels were significantly increased with cromakalim and glibenclamide. However no significant alteration in serum T_3 levels was observed with pinacidil and KRN 2391. Serum TSH levels were significantly decreased with pinacidil. No significant alteration was observed in serum TSH levels by other potassium channel modulators. Serum cortisol levels were significantly decreased with all the three potassium channel openers while glibenclamide did not produce any significant change in serum cortisol levels. Our data suggest that potassium channels may be involved in the release of

***For correspondence**

E-mail: dranitalmcp@rediffmail.com

hormones other than insulin. It also indicates that there is involvement of different types of potassium channels in the action of different potassium channel modulators.

The role of potassium channels in regulating the membrane potential, excitability of cells and in various physiological processes is well-established[1]. These channels are ubiquitous and exist as several subtypes, with multiple subtypes often being present on a single cell[2]. Role of potassium channels in the release of insulin has been well established. Potassium channel blockers like glibenclamide, glipizide and glimpiride, which are the first line therapy in the treatment of Type II Diabetes Mellitus, act by closure of adenosine triphosphate sensitive potassium channels (K_{ATP})[3,4]. Diazoxide, pinacidil, cromakalim and nicorandil used in cardiovascular disorders are reported to activate K_{ATP} Channels in insulin secreting cells of pancreas. Whereas, minoxidil, although a potassium channel opener is reported to potentiate glucose stimulated insulin release in pancreatic beta cells[5]. As in the release of insulin, intracellular calcium concentration is the key internal regulator in the secretion of other hormones also. Calcium channel blockers can reduce endocrine hormone release[6,7]. Hyperpolarisation induced by potassium channel openers prevent calcium entry through voltage operated channels and thus act as indirect calcium entry blockers and be expected to have a pharmacological profile similar to that of the calcium channel blockers[8]. In our earlier studies, the short-term treatment with pinacidil and cromakalim was found to produce a significant fall in serum cortisol levels in rats and dogs[9,10]. In view of these facts, the objective of our present investigation was to study the effect of chronic treatment with pinacidil, cromakalim, KRN 2391and glibenclamide on levels of various hormones like insulin, T_3, TSH and cortisol in rats.

Male Wistar rats (200-250 g), obtained from Cadila Pharmaceuticals, Ahmedabad were used for the study. Animals were housed at ambient temperature (21+1°) and relative humidity (55+5%) with fixed 12 h light/dark cycles and free access to food and water. At least 7 days were allowed for adaptation before the animals were used for the experiments. The experimental protocol was approved by the Institutional Animal Ethical Committee of L. M. College of Pharmacy. Rats were randomly divided into five groups of six animals each. Animals of group II to IV were treated orally with pinacidil, KRN 2391 or cromakalim respectively at the dose of 200 µg/kg for 30 d. Animals of fifth group were treated with potassium channel blocker glibenclamide (20 mg/kg). Pinacidil and cromakalim were administered as aqueous solutions, whereas KRN 2391 and glibenclamide were suspended in water using (0.5%, w/v) sodium carboxymethylcellulose. Group I (control) received equivalent volume of vehicle. Blood samples were collected on 30th day by tail snipping method and analyzed for serum glucose by colorimetric analysis and for serum insulin, T_3 and TSH levels using radioimmunoassay kits obtained from Bhabha Atomic Research Center, Mumbai. Cortisol levels were analyzed in plasma by radioimmunoassay (Diagnostic systems, Texas, U.S.A.). Results were analyzed using a one-way analysis of variance followed by Tuckey's test. The value of p less than 5% ($p< 0.05$) was considered as significant.

Serum insulin levels were found to be significantly decreased in animals treated with cromakalim whereas pinacidil and glibenclamide treated animals showed significant higher serum insulin levels as compared to control. KRN 2391 had no effect on serum insulin levels. Glucose levels were found to be significantly decreased only with glibenclamide. No significant change was observed in serum glucose levels by any of the potassium channel openers. Serum T_3 levels were significantly increased with cromakalim and glibenclamide. However no significant alteration in serum T_3 levels was observed with pinacidil and KRN 2391. Serum TSH levels were significantly decreased with pinacidil. However cromakalim, KRN 2391 and glibenclamide showed no significant change in serum TSH levels. Serum cortisol levels were significantly decreased by all the three potassium channel openers while no change was observed with glibenclamide (Table 1).

The results of present study show that cromakalim treatment for 30 days produces significant decrease in serum insulin levels with no significant alteration in serum glucose levels. This finding supports the previous report by Dunne *et al.*[11] that effect of cromakalim on K_{ATP} channels is far less impressive than diazoxide and thus cromakalim may prove to have little or no risk of hyperglycemia as a side effect when used as an antihypertensive agent. There appears to be differences among potassium channel openers with respect to their effectiveness against K_{ATP} channel since pinacidil was found to produce significant increase in serum insulin levels like glibenclamide. This result is similar to that of minoxidil which was reported to significantly potentiate glucose-induced insulin release by inhibiting K_{ATP}

TABLE 1: EFFECT OF POTASSIUM CHANNEL MODULATORS ON HORMONE LEVELS

Parameters	Treatment				
	Control	Pinacidil	KRN 2391	Cromakalim	Glibenclamide
Insulin (IU/ml)	25.2±2.34	113.4±24.5*	22.8±4.57	14.3±2.47*	117.6±20.6*
Glucose (mg/dl)	87.8±6.30	93.0±8.50	107.6±5 .76	98.2±1.25	61.5±5.80*
T3 (ng/ml)	1.12±0.09	1.35±0.13	1.25±0.06	1.46±0.01*	1.83±0.18*
TSH (mIU/ml)	0.29±0.03	0.20±0.002*	0.22±0.03	0.26±0.03	0.28±0.02
Cortisol (μg/dl)	0.43±0.02	0.33±0.01*	0.29±0.001*	0.32±0.002*	0.42±0.01

All values are expressed as mean S.E.M., (n=6), * significantly different from control at $p< 0.05$.

channels[5]. Variation in the effect of potassium channel openers on serum insulin levels is further apparent from the findings of our study with another potassium channel opener KRN 2391 since it did not produce any significant alteration in serum insulin and glucose levels.

Increase in serum T_3 levels observed may be because of decrease in intracellular Ca^{+2} concentration by potassium channel openers since it is reported that increase in intracellular Ca^{+2} significantly decreases iodide accumulation response to TSH by thyroid cell[12]. Significant decrease in serum TSH levels by pinacidil, may be due to negative feedback provided by increase in serum T_3 levels. It has also been reported that activation of inward rectifying potassium current in pituitary gland by somatostatin hyperpolarizes the membrane and causes reduction in TSH secretion[13]. It is possible that the reduction in TSH levels by pinacidil may also be due to activation of this channel in pituitary gland. The decrease in plasma cortisol levels observed in the present investigation by potassium channel openers may be because of calcium dependent mechanisms reported to be responsible for the release of substances from secretory organs[6].

In conclusion, our data suggests that potassium channels may be involved in the release of hormones. The effects of potassium channel modulators are variable and did not show a well-defined pattern. This may be due to differences in the structure or control mechanisms of potassium channels (even of the same type) in various tissues.

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