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L6 skeletal muscle cells have functional V_1-vasopressin receptors coupled to stimulated inositol phospholipid metabolism

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The effects of vasopressin and related peptides upon the rat skeletal muscle cell line, L6, have been examined. No effects upon cellular cyclic AMP levels were found indicating that L6 cells possess no functional V_2-vasopressin receptors. Vasopressin and its analogues did, however, stimulate the rapid and dose-dependent accumulation of inositol phosphates. This effect and the rank order of potency of vasopressin analogues demonstrate the presence of functional V_1-vasopressin receptors upon L6 cells. These results suggest that the L6 line may be a useful model for vasopressin effects upon skeletal muscle metabolism.

Vasopressin; V_1 receptor; Inositol phosphate; (L6 skeletal muscle cell)

1. INTRODUCTION

Vasopressin has actions both as a hormone (anti-diuretic hormone) and as a neural peptide transmitter in various tissues. It appears to act through two distinct types of receptors which have been termed V_1 and V_2 receptors, both of which are coupled to distinct second messenger generation systems [1]. V_1 receptors are coupled to the stimulation of inositol phospholipid breakdown whereas V_2 receptor occupancy leads to the stimulation of adenylate cyclase activity.

From in vivo studies with various rat skeletal muscle preparations [2] and in vitro studies using chick embryonic skeletal muscle cells in culture [3] it has been proposed that vasopressin triggers the breakdown of muscle glycogen by a mechanism involving the activation of glycogen phosphorylase, the activity of this enzyme apparently being augmented by two distinct pathways, namely by an increase in either intracellular free Ca^{2+} or cyclic AMP concentrations.

In hepatocytes, vasopressin activates glycogen phosphorylase in a Ca^{2+}-dependent, cyclic AMP-independent manner [4]. This effect of the peptide upon Ca^{2+} levels is, however, secondary to the stimulated breakdown of phosphatidylinositol 4,5-bisphosphate and the consequent production of the two second messengers, inositol 1,4,5-trisphosphate and 1,2-diacylglycerol (reviews [5,6]). The mechanism whereby vasopressin acts upon muscle tissue is, however, unknown and there are no reports in the literature as to ligand-stimulated effects upon inositol phospholipid metabolism in skeletal as opposed to smooth muscle cells.

Accordingly, we have examined the effects of vasopressin upon the L6 skeletal muscle line in order to investigate the presence and characteristics of vasopressin receptors on a differentiated cell line model of skeletal muscle. Here, we show that the L6 skeletal muscle line possesses

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a functional vasopressin V_1-receptor which is coupled to the stimulation of inositol phospholipid breakdown.

2. MATERIALS AND METHODS

L6 cells, purchased from the American Type Culture Collection, were cultured in 10 cm diameter plastic tissue culture dishes in Dulbecco's modified Eagle's medium containing 10% (v/v) heat-inactivated foetal calf serum and 100 μg/ml gentamycin. The cells were maintained at 95% humidity at 37°C in an atmosphere of 92% air, 8% CO_2. 48 h before experiments were performed the medium was changed to one containing 1 μCi/ml *myo*-[2-^{3}H]inositol (NEN). Cells were 75–80% confluent when used for experiments.

On the day of experiments the medium was removed from the dishes and the monolayers washed with Hank's buffered saline containing 1% (w/v) bovine serum albumin and 10 mM glucose (HBG). The cells were then scraped from the dishes using a teflon-coated spatula, washed in HBG and resuspended in HBG containing 10 mM LiCl. The cell suspension (0.24 ml, 2×10^6 cells/ml) was dispensed into plastic biovials containing 10 μl of test substance or buffer. The vials were gassed (95% O_2:5% CO_2) capped and incubated in a shaking water bath at 37°C for 45 min. Incubations were terminated by the addition of 0.94 ml chloroform/methanol (1:2) and the phases split by the addition of 0.31 ml each of chloroform and water. Radioactivity associated with the total inositol phosphates in an aliquot of the aqueous layer was determined by liquid scintillation counting following batch chromatography on Dowex 1×8 formate resins as in [7]. The radioactivity in the lipids was determined upon a dried aliquot of the lower, organic phase.

Cyclic AMP was determined in cells by use of a binding protein assay [8]. Experiments were performed using cells grown on 35 mm diameter dishes. The growth medium was aspirated and replaced with HBG and the cells allowed to incubate for 45 min. IBMX was then added to a final concentration of 1 mM. After 15 min stimulants were added as described in section 3. Incubations were terminated by the addition of 0.5 ml ice-cold 5% perchloric acid and the cyclic AMP concentrations determined as described in [8].

3. RESULTS AND DISCUSSION

Table 1 shows that [^{3}H]inositol-labelled phosphates were generated in L6 muscle cells upon stimulation by the addition of vasopressin. This effect was observed in response to arginine-vasopressin, lysine-vasopressin, vasotocin and oxytocin, but not in response to mesotocin or pressinoic acid. Table 1 also shows that arginine-vasopressin did not stimulate the production of inositol phosphates in the presence of the vasopressin receptor antagonist, 1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)-2-(*O*-methyl)tyrosine]-8-arginine-vasopressin. The effects of vasopressin upon inositol phospholipid metabolism can therefore be concluded to be receptor mediated.

Stimulation of L6 cells with arginine-vasopressin had no effect upon the intracellular concentration of cyclic AMP. Stimulation of the cells with adrenaline, however, caused a greater than 1000-fold increase in cyclic AMP levels (not shown). This result confirms previous observations

Table 1

The effect of vasopressin and analogues upon inositol phosphate generation in L6 cells

Agonist	cpm in inositol phosphates	Fold increase over control
None	308 ± 34	–
Lysine-vasopressin	1447 ± 124	4.7 ± 0.5
Arginine-vasopressin	1210 ± 124	3.9 ± 0.6
Vasotocin	1327 ± 73	4.3 ± 0.4
Mesotocin	354 ± 64	1.2 ± 0.4
Oxytocin	527 ± 38	1.7 ± 0.2
Pressinoic acid	274 ± 37	0.9 ± 0.1
Arginine-vasopressin + antagonist	275 ± 15	0.9 ± 0.1

L6 cells were incubated and labelled as described in section 2. The cells were washed, incubated with agonists for 45 min in the presence of 10 mM LiCl and the radioactivity in inositol phosphates determined as described in section 2. Results are means ± SD from one experiment typical of two; $n = 3$ in each case. Concentrations in each case are 40 μM except for arginine vasopressin which is 4 μM, or 400 nM when tested against the antagonist

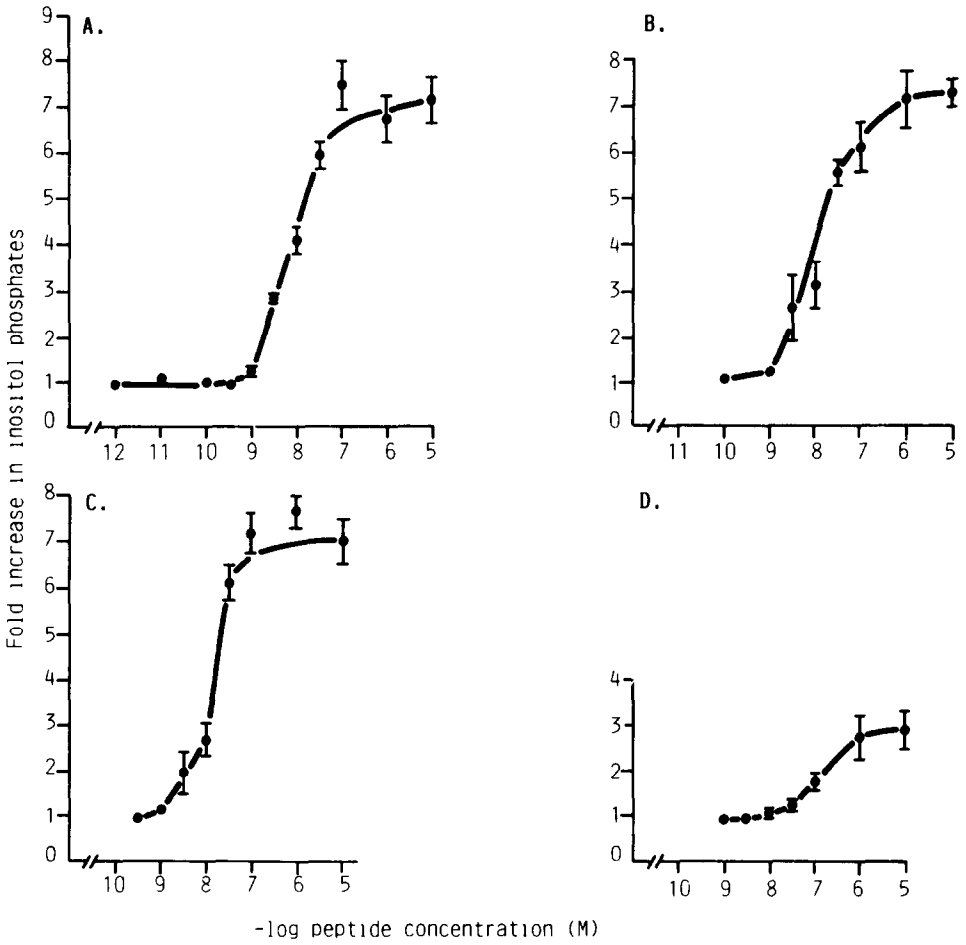

Fig.1. Dose-response curves for the effects of various vasopressin analogues upon inositol phosphate generation in L6 cells. Experiments were performed as described in the legend to table 1 and section 2. The results are expressed as means ± SD of the fold increase in cpm in the inositol phosphates compared to control values. Typically control inositol phosphates were 150–300 cpm and the control lipids 3000–5000 cpm. The data are pooled from 12 separate experiments and n = 6–24. A, arginine-vasopressin; B, lysine-vasopressin; C, vasotocin; D, oxytocin.

which demonstrated functional β-adrenergic receptors in L6 skeletal muscle cells [9].

Since V_1 receptors have been shown to be coupled to stimulated inositol phospholipid breakdown and V_2 receptors to the stimulation of adenylate cyclase activity in a variety of tissues (see [4] and references therein) it is clear that L6 skeletal muscle cells possess functional V_1-vasopressin receptors coupled to stimulated inositol phospholipid metabolism. The nature of this vasopressin receptor is confirmed by the rank order of potency obtained from the results presented in fig.1. Thus, arginine-vasopressin = lysine-vasopressin > vasotocin > oxytocin ≫ mesotocin = presinoic acid, the last two peptides being inactive. In addition the ED_{50} for arginine-vasopressin of 10 nM (fig.1A) compares well with published values of 5 and 21 nM for vasopressin-stimulated inositol phosphate production [10] and inositol phospholipid breakdown [11], respectively, in isolated hepatocytes. An identical ED_{50} value to that of arginine-vasopressin was found for lysine-vasopressin (fig.1B). Vasotocin (fig.1C) has an ED_{50} of 20 nM whereas that for oxytocin was

found to be 200 nM (fig.1D). An identical rank order of potency was found in isolated hepatocytes, a V_1-vasopressin receptor containing cell, by Kirk et al. [4].

The rank order of potency demonstrates that for stimulation of inositol phospholipid breakdown a basic amino acid is required at position 8 of the agonist peptide. The discrimination between oxytocin and mesotocin is surprising in that these two peptides differ only at position 8, where oxytocin has a leucine and mesotocin an isoleucine residue. This result strongly suggests a precise spatial requirement for the side chain in position 8 in that isoleucine differs from the acceptable substitutions, lysine, arginine and leucine, in having a methyl group on the α-carbon position.

It has been proposed previously that vasopressin exerts effects upon skeletal muscle glycogen metabolism [2,3]. The results presented here imply that such a function is mediated by stimulation of inositol phospholipid metabolism by functional V_1-vasopressin receptors. Since the primary regulation of skeletal muscle metabolism and function is neuronal, it is probable that any physiological regulation of glycogen metabolism by vasopressin is also of neuronal origin and may be related to the occurrence of a vasopressin-like peptide (VLP) in peripheral nerves [12]. Indeed, VLP has been suggested to be active preferentially at V_1 receptors.

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