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Category No. 2 | Video Demonstration | Ref. No. 305

Abstract Reproduction Form B-1

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Title (type in CAPITAL LETTERS): MECHANISMS FOR INHIBITION OF RAT BLADDER SMOOTH MUSCLE CONTRACTION BY ETHANOL (ETOH)

Aim of study

Acute ethanol intoxication produces urinary retention in patients with bladder outlet obstruction(BOO), which suggests that EtOH has an inhibitory effect on bladder smooth muscle contraction. Therefore, We investigate here mechanisms for inhibition by EtOH of bladder smooth muscle by measuring contraction of rat bladder smooth muscle strips and [$^{45}Ca^{2+}$] influx into rat bladder smooth muscle in primary culture.

Methods

Bladder muscle strips derived from rats were used examine effects of EtOH(1-100mM) on smooth muscle contraction induced by 126 mM KCl in Tyrode's solution. The contraction was mesured by an isometric transducer. Influx of [$^{45}Ca^{2+}$] was examined using primary culture of rat bladder smooth muscle cells prepared from rat bladder dome and base. After preincubation of cells with Ca^{2+}-free KRBH at 37℃ for 10 min, the cells were incubated in Kreb's ringer bicarbonate buffer containing 20 mM HEPES(KRBH;pH 7.4), at 37℃ for 2 min with or without EtOH and various drugs. The reaction was initiated by adding 1.26 mM [$^{45}Ca^{2+}$]Cl2, and the termination of the reaction was carried out by rapid aspiration of KRBH containing [$^{45}Ca^{2+}$] followed by five times washing with ice-cold KRBH. An aliquot of alkaline digested cells was subjected to mesure [$^{45}Ca^{2+}$] influx byliquid scintilation spectrometory.

Results

1.Effects of EtOH on concentration of muscle strips induced by KCl: EtOH dose-dependently inhibited KCl-induced contraction of muscle strips, and its maximal inhibition was observed at 50mM, suggesting that EtOH has an inhibitory action on bladder smooth muscle contraction.
2.Effects of EtOH on [$^{45}Ca^{2+}$] influx into smooth muscle cells: KCl dose- and time-dependently increased [$^{45}Ca^{2+}$] influx, KCl (50 mM)-induced [$^{45}Ca^{2+}$] influx was significantly inhibited by tetrodotoxin, dibucaine, procainamide and verapamil, indicating that the KCl-induced [$^{45}Ca^{2+}$] influx into smooth muscle cells occurs through L-type VDCCs following depolarization of the cells. Bay k 8644, a L-type VDCC activator, also dose-dependently increased the influx. KCl (50 mM)-induced [$^{45}Ca^{2+}$] influx in both types of cells was dose-dependentry inhibited by EtOH(Fig.1). In addition, [$^{45}Ca^{2+}$] influx induced by both KCl and Bay k 8644 was completely abolished by 50 mM EtOH(Fig.2).

Conclusions

These results descrived above indicate that the inhibition of KCl-induced contraction of muscle strips by EtOH may be mediated via the suppression of Ca^{2+} influx through L-type VDCCs by EtOH.

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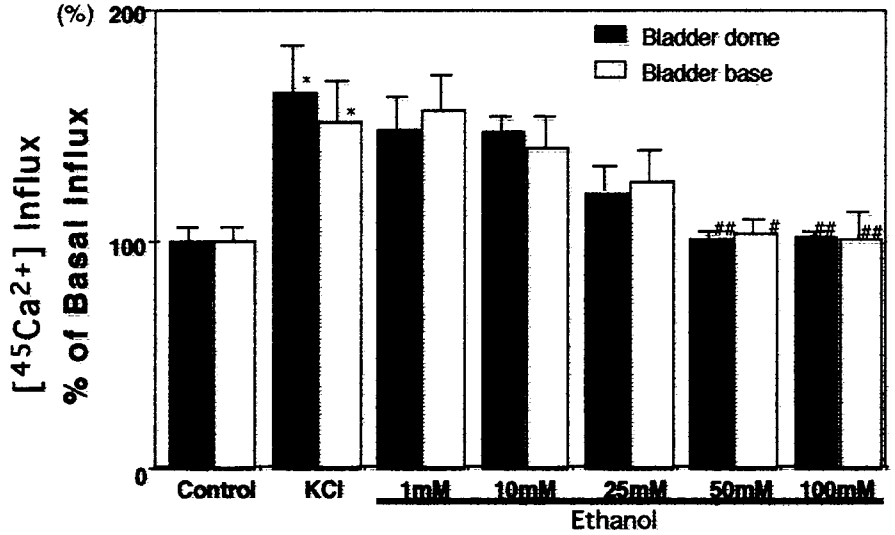

Fig.1 Comparative study of bladder body and base with [$^{45}Ca^{2+}$] influx with 50mM ethanol . Each value represents the mean±S.E.M.Basal influx of dome and base were 203.8±9.9 , 203.4±9.6 cpm/protein/3min, respectively. **p<0.01,compared with the basal influx (Bonferroni's test , N=4). #p<0.05 and ##p<0.01, compared with thevalue determined in the presence of 50mM KCl alone (Dunnett's test , N=4).

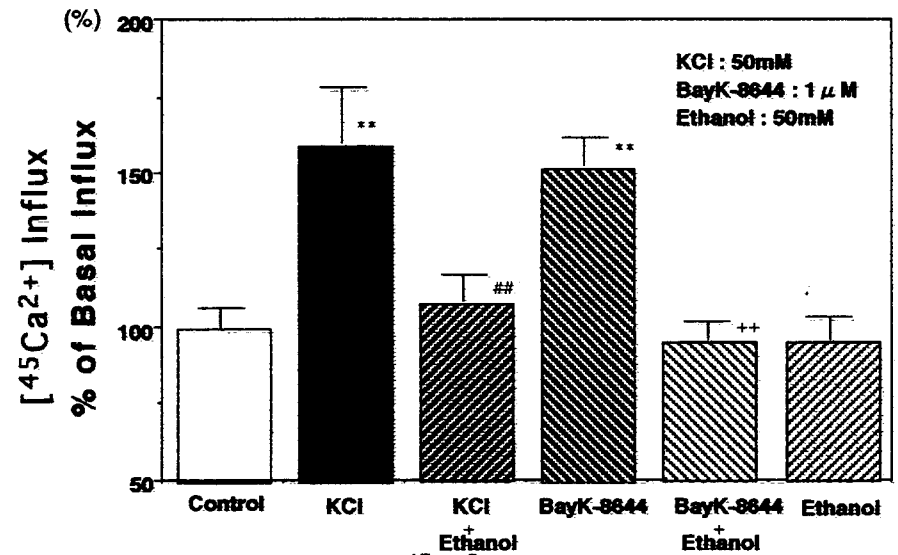

Fig.2 Direct effect of Ethanol (50mM) to [$^{45}Ca^{2+}$] influx by KCl and voltage-dependent L type Ca^{2+} channel agonist Bay K 8644 into primary cultured bladder smooth muscles. Each value represents the mean±S.E.M. **p<0.01, compared with the basal influx (Bonferroni's test, N=4). ##p<0.01, compared with the value determined in the presence of 50mM KCl alone (Dunnett'stest , N=4).++p>0.01, compared with the value determined in the presence of 1 μM Bay K 8644 alone (Dunnett's test , N=4).