



Title	Preparation of a Panel of M13 Phages Reconizing Influenza Virus Proteins
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Effects of glucocorticoids on catecholamine secretion and electrical activity of guinea-pig adrenal chromaffin cells

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1. Effects of cortisol, a natural glucocorticoid, and dexamethasone, a synthetic one, on secretagogues-induced catecholamine (CA) secretion were investigated in perfused guinea pig adrenal glands. The effects of glucocorticoids on the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{in}}$) and ionic channel currents were also examined in isolated adrenal chromaffin cells.
2. Cortisol (10–300 μM) and dexamethasone (10–300 μM) produced dose-dependent inhibition of nicotine (20 μM)-induced CA secretion with the apparent IC_{50} values of 132 μM and 30 μM , respectively.
3. Cortisol (10–300 μM), but not dexamethasone (10–300 μM), inhibited muscarine (20 μM)-induced CA secretion with an apparent IC_{50} value of 169 μM . The increase in $[\text{Ca}^{2+}]_{\text{in}}$ evoked by muscarine (20 μM) was not affected by both glucocorticoids.
4. High K^+ (56 mM)-induced CA secretion was not affected by both glucocorticoids. Dexamethasone, but not cortisol, slightly depressed an increase in $[\text{Ca}^{2+}]_{\text{in}}$ evoked by high K^+ .
5. In whole cell voltage-clamped cells, voltage-dependent Na^+ currents evoked by depolarizing pulses were not affected by both glucocorticoids. Dexamethasone, but not cortisol slightly depressed the Ba^{2+} currents through voltage-dependent Ca^{2+} channels.
6. At a holding potential of -70 mV, nicotine (10–100 μM) caused transient inward currents in a dose-dependent manner. The dose-response curve for nicotinic currents was downwardly shifted by cortisol (30 μM) and dexamethasone (10 and 30 μM). Nicotine (50 μM)-evoked inward currents were dose-dependently inhibited by cortisol (3–100 μM) and dexamethasone (3–100 μM) with the apparent IC_{50} values of 132 μM and 30 μM , respectively.
7. These results indicate that glucocorticoids have short-term inhibitory effects on nicotine-induced CA secretion probably by the inhibition of nicotinic receptor channels in guinea-pig adrenal chromaffin cells. The inhibitory action of cortisol on secretory response to muscarine remained to be clarified.

Preparation of a Panel of M13 Phages Reconizing Influenza Virus Proteins

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Influenza A viruses distribute among a variety of animals including humans, pigs, and birds.

Pigs act as the intermediate host generating genetic reassortants between human and avian

viruses in the epithelial cells lining their upper respiratory tract. One of these reassortants, possessing new subtype of the hemagglutinin (HA) derived from avian virus, that infect, transmit to, and cause pandemic influenza in humans, is defined as a new pandemic strain. Since the HA is the target antigen for neutralizing antibodies, prediction of or immediate identification of the HA subtype of a new pandemic strain is important for the prevention and control of emerging influenza.

The phage display system was employed for

the detection of influenza A virus proteins. In the present study, phage clones that specifically bind to purified influenza virus were selected from the library. The phage clones that bound to the nucleoprotein (NP) or the matrix protein were obtained. However, none of the clones bound to the HA. Some clones recognized both influenza A and B virus NPs. Such phage clones may be useful for the detection both of influenza A and B viruses from nasopharyngeal samples of patients and animals for rapid diagnosis of influenza virus infection.

Amino Terminus of Bovine Herpesvirus 1 Glycoprotein gB is Responsible for Membrane Fusion Activity

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Herpesviruses penetrate into host cells by fusion of virus envelope with cell membrane. Since the glycoprotein gB homologues are conserved among all herpesviruses, the glycoprotein is believed to play an important role in the process of virus infection.

To provide information on the functional domain of bovine herpesvirus 1 (BHV1) gB, escape mutant resistant to anti-gB monoclonal antibody 185/1, which required complement for virus neutralization, was selected. Nucleotide sequencing analysis revealed that amino acid substitution from Arg to Gln occurred at the amino terminal of the mutant gB. This substitution did not influence binding of virus to heparin, analog of viral receptor, kinetics of adsorption to and penetration into host cells, or yield of progeny viruses. On the other hand, the mutant virus formed smaller plaques than those of wild type

virus. This finding indicates that the amino acid substitution at the amino terminal of gB reduced efficiency of cell-to-cell spread of the virus.

The gB-specific complement-independent neutralizing monoclonal antibody 118E inhibited the enlargement of plaques of the wild type virus. Since antibody 118E lacked reactivity to the escape mutant, the antibody should bind to the epitope overlapping that of antibody 185/1. These results indicate that cell-to-cell spread as well as initiation of infection of BHV1 is interfered with by binding of the antibody to the amino terminal of gB.

Taken together, the present results suggest that the amino terminal region of gB are responsible for membrane fusion activity of the glycoprotein which play roles in penetration and cell-to-cell spread of BHV1.