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Stock solutions of cocaine, isradipine, nomifensine, lidocaine, DHβE, CP8, L-741,626, and water-soluble cholesterol

In 1 collection

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We use this protocol and it's working

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Abstract

This protocol details the making of the following stock solutions used in **Brimblecombe, K.R. et al. (2023)**:**cocaine** (inhibits the activity of dopamine transporters (DATs)), **isradipine** (inhibits L-type voltage-gated Ca^{2+} channels (LTCC)), **nomifensine** (inhibits monoamine re-uptake), **lidocaine** (blocks voltage-gated Na^{+}- channels), **dihydro-β-erythroidine (DHβE)** (nicotinic acetlycholine receptor (nAChR) antagonist), **1-(3-chlorophenethyl)-3-cyclopentylpyrimidine-2,4,6-(1H,3H,5H)-trione (CP8)** (a potent and highly selective $Ca_V1.3$ L-type calcium channel antagonist; see **Protocol: Synthesis of 1-(3-chlorophenethyl)-3-cyclopentylpyrimidine-2,4,6-(1H,3H,5H)-trione (CP8)**), **L-741,626** (inhibits D2 receptors), and **water-soluble cholesterol** (DAT function in *Snca*-null mice is augmented, see **Threlfell et al., 2021**).

Guidelines

Drug concentrations were chosen in accordance with previous studies (**Acevedo-Rodriguez et al., 2014**; **Brimblecombe et al., 2015**).

Materials

Drugs:

- Dihydro-β-erythroidine (DHßE) (M_r 365.27, stock powder stored at room temperature)
- Isradipine (M_r 371.39, stock powder stored at 5°C)
- Cocaine (Torcis, M_r 339.82, stock powder stored at room temperature)
- Nomifensine (maleate salt) (M_r 354.4, stock powder stored at room temperature)
- Lidocaine (M_r 234.34, stock powder stored at room temperature)
- CP8 (M_r 334.8, stock powder stored at room temperature)
- L741-626 (M_r 340.85; stock powder stored at room temperature)

Controls:

- **Water-soluble Cholesterol** (Sigma Aldrich, #SKU C4951-30MG, stock powder stored at -20°C)
- Me-ß-cyclodextrin (M_r ~1320, stock powder stored at room temperature)

Solvents:

- Dimethylsulfoxide (DMSO)
- Ethanol
- 0.1M Hydrocloric Acid (HCl)
- dH2O

Troubleshooting

Cocaine (5 µM)

1 Make 50 mL aliquots of 10 mM stock solution:
- add 3.4 mg per 1 mL dH_2O
- store at -20°C

Note

Controlled substance! Stored in locked cabinet. Keep log book up to date.

2 For each experiment, dilute 50 µL of 10 mM stock solution (one aliquot) in 100 mL of bicarbonate-buffered artificial cerebrospinal fluid (aCSF) solution for a final working concentration of 5 µM.

Isradipine (5 µM)

3 Make 50 µL aliquots of 10 mM stock solution:
- add 2.69 mL DMSO to 10 mg powder
- stored at -20°C

4 For each experiment, dilute 50 µL of 10 mM stock solution (one aliquot) in 100 mL aCSF for final working concentration of 5 µM.

Nomifensine (10 µM)

5 Make 100 µL aliquots of 10 mM stock solution:
- add 3.54 mg per 1 mL 0.1 HCl
- stored at -20°C

6 For each experiment, dilute 100 µL of 10 mM stock solution (one aliquot) in 100 mL aCSF for final working concentration of 10 µM.

Lidocaine (100 µM)

7 Make 100 µL aliquots of 100 mM stock solution:
- add 2.34 mg per 1 mL ethanol
- stored at -20°C

8 For each experiment, dilute 100 µL 100 mM stock solution (one aliquot) in 100 mL aCSF for final working concentration of 100 µM.

Dihydro-β-erythroidine (DHβE)(1 µM)

9 Make 0.5 mL aliquots of 10 mM stock solution:
- add 2.8 mL dH_2O to 10 mg powder
- store at -20°C

10 Make 100 µL aliquots of 1 mM working stock solution:
- add 4.5 mL dH2O to 0.5 mL 10 mM stock solution
- store at -20°C

11 For each experiment, dilute 100 µL of 1 mM working stock solution (one aliquot) in 100 mL of aCSF solution for a final working concentration of 1 µM.

CP8 (10 µM)

12 Make 100 µL aliquots of 10 mM stock solution:
- add 3.35 mg per 1 mL DMSO
- store at -20°C

13 For each experiment, dilute 100 µL 10 mM stock solution (one aliquot) in 100 mL aCSF for final working concentration of 100 µM.

L741-626 (1 µM)

14 Make 100 µL aliquots of 10 mM stock solution:
- add 2.93 mL DMSO to 10 mg powder
- store at -20°C

15 For each experiment, dilute 10 µL 10 mM stock solution (one aliquot) in 100 mL aCSF for final working concentration of 1 µM.

Water-soluble Cholesterol (50 µg/mL)

16 Make 45 mg of cholesterol per gram:
- add 11.1 mg water-soluble cholesterol to aCSF

Note

Contains 1 mM Me-ß-cyclodextrin to make it water soluble. Therefore, control experiments are conducted in 1 mM Me-ß-cyclodextrin as a vehicle control.

Me-ß-cyclodextrin (1000 µM) - Control Solution

17 Make 20 µL aliquots of 0.5 M stock solution:
- add 655 mg per 1 mL dH_2O [Yes it really is this soluble!]
- store at 4°C

18 For each experiment dilute 20 µL 0.5M stock solution (one aliquot) in 10 mL aCSF for final working concentration of 1 mM.