FIGURE LEGENDS (SUPPLEMENTARY DATA)

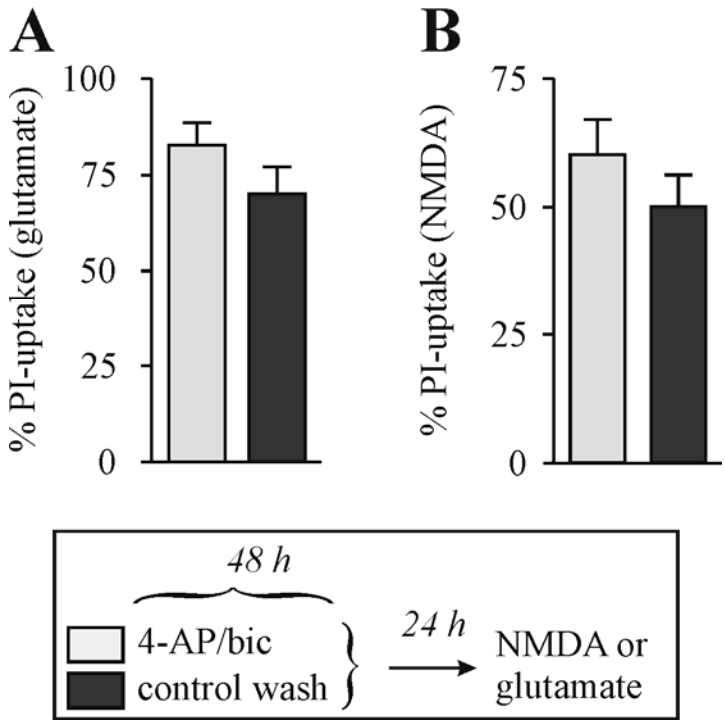
Figure 1. 4-AP/bic treatment does not precondition against NMDA or glutamate toxicity.
Cortical neuron cultures were subjected to 'low' 4-AP/bic preconditioning (light bars) or control wash (dark bars), followed by exposure to an excitotoxin 24 h later, with assessment of the % PI-uptake accomplished 24 h later. Excitotoxins employed were **(A)** 25 μM glutamate for 15-20 min; or **(B)** 30 μM NMDA for 15-20 min. Preconditioning did not result in a significant suppression of the % PI-uptake, as assessed using a Student's t-test ($P > 0.05$).

Figure 2. 4-AP/bic treatment preconditions against delayed, but not acute, staurosporine toxicity. Cultures were subjected to bicuculline (bic; 50 μM), 'high' (2.5 mM 4-AP + bic) or 'low' (50 μM 4-AP + bic) 4-AP/bic preconditioning (light bars) or control wash (dark bars), with an apoptotic agent, staurosporine (0.75 μM for 24 h) treated at different times, followed by measurement of the % PI-uptake. Specific conditions were as follows: **(A)** Bic, 4-AP/bic or control wash combined with staurosporine for 24 h; **(B)** Bic, 4-AP/bic or control wash applied for 48 h, with staurosporine present between the 24-48 h time point; **(C)** Bic, 4-AP/bic or control wash applied for 48 h, followed by exposure to staurosporine for 24 h. Brackets denote a significant difference ($p < 0.05$) between indicated conditions using an ANOVA.

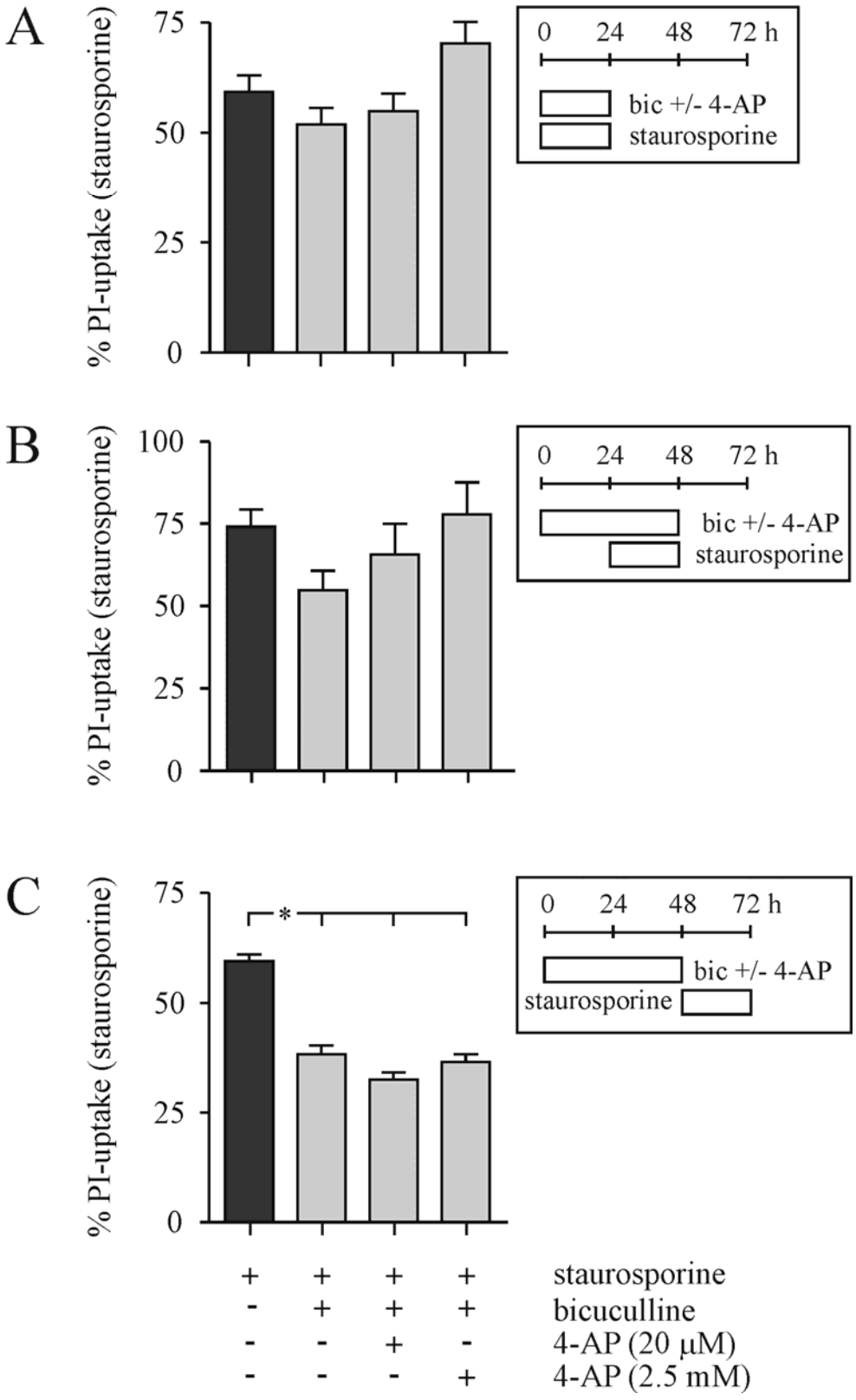
Figure 3. Effect of additional receptor antagonists or protein kinase inhibitors on 'low' 4-AP/bic preconditioning-induced OGD tolerance. Cortical neuron cultures were subjected to 48 h 'low' 4-AP/bic preconditioning (light bars) or control wash (dark bars) in the absence (labeled antagonist-free) or presence of a receptor antagonist or protein kinase inhibitor, followed by exposure to 65-80 min OGD 24 h later, and then measurement of the %-PI uptake measured 24 h later. Antagonists examined were an NMDA receptor antagonist, MK-801 (1 μM; n = 12); a K_{ATP} channel inhibitor, glybenclamide (20 μM; n = 12-15) or tolbutamide (25 μM; n = 12); an adenosine A_1 receptor antagonist, 8-cyclopenthyltheophylline (8-CPT; 30 μM; n = 6-12); or inhibitors of PKA, H-89 (5 μM; n = 12), PKA inhibitory peptide (14-22) (10 μM; n = 12) or KT 5720 (10 μM; n = 12). Brackets denote a significant difference ($p < 0.05$) between indicated conditions using an ANOVA.

Figure 4. Prolonged elevations of neuronal pCREB levels observed during tolerance-inducing preconditioning.
(A) Representative immunoblots showing expression of pCREB and CREB from protein samples collected immediately after exposure of cultures to 'high' 4-AP/bic for 20 min, 1 h or 48 h, as well as for protein samples collected from cultures which had been exposed to 4-AP/bic or control wash for 48 h and then washout for 20 min, 1 h or 48 h. **(B)** Representative immunoblots showing expression of pCREB and CREB from protein samples collected 1 h after exposure of cultures exposed to 'high' 4-AP/bic for 4 h. **(C)** Representative immunoblots showing expression of pCREB and CREB from protein samples collected immediately after exposure of cultures to 'low' 4-AP/bic for 6 h or 48 h, or from samples collected 4 h or 24 h after 4-AP/bic washout. In all immunoblots, pCREB represents CREB phosphorylated at Ser^{133} (upper panel). pCREB gels were stripped and re-probed for CREB (lower panel). Immunoblots are representative of 4 experiments from 4 different platings, with each experiment comprised of 2-3 wells/condition. **(D)** Same-field immunofluorescence images of cultures labeled with an antibody to MAP-2 or pCREB, following 6 h exposure to 'high' 4-AP/bic. This image is representative of images acquired from 3 different platings, comprised of 3 coverslips/condition with a minimum of 3 images acquired per coverslip.

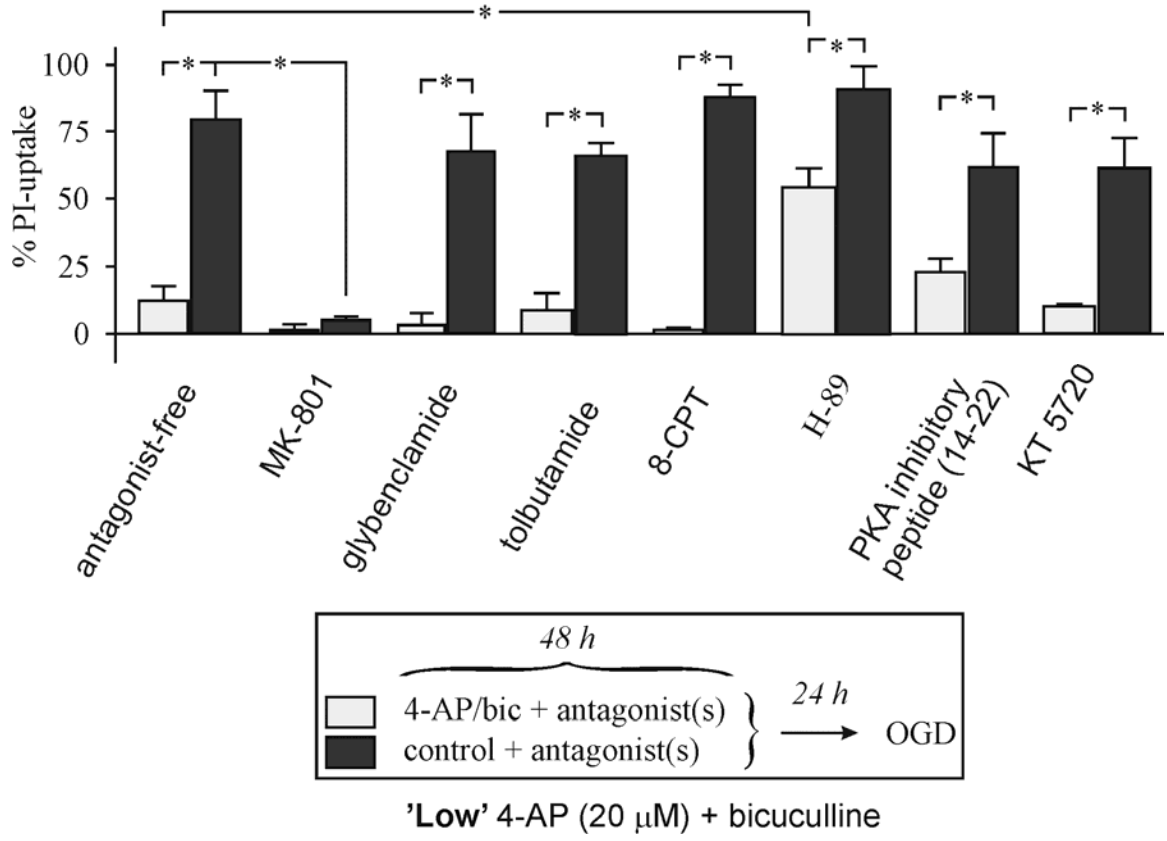
Supplementary Fig. 1



Supplementary Fig. 2



Supplementary Fig. 3



'Low' 4-AP (20 μM) + bicuculline

Supplementary Fig. 4