Supplemental Data

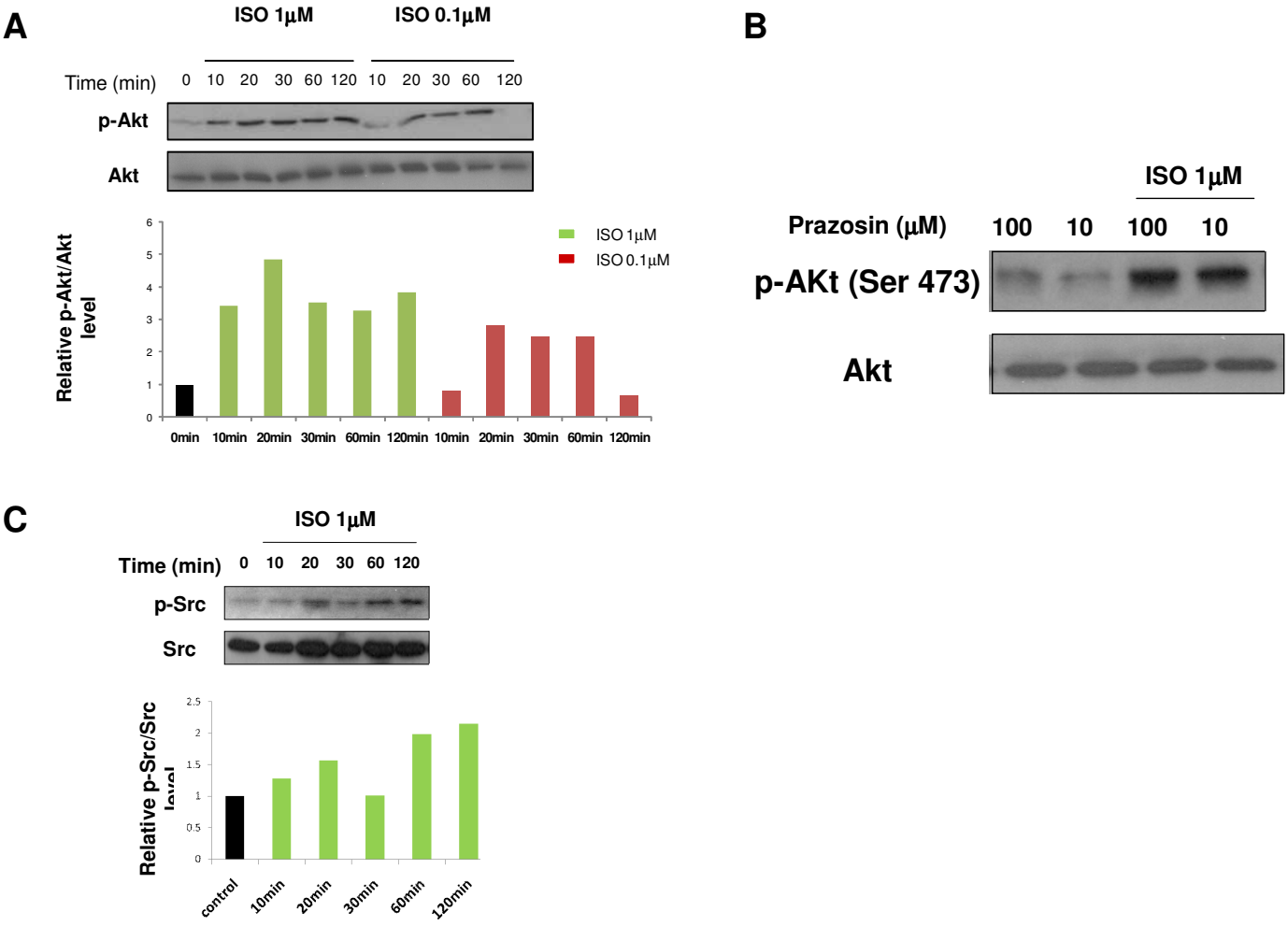

Figure I A) Neonatal rat cardiac myocytes were stimulated with indicated concentrations of isoproterenol (ISO) for the indicated durations. Levels of phospho-Ser 473 Akt and total Akt were determined by immunoblotting and densitometric analyses. In the lower panel, a bar graph of relative phospho-Akt/total Akt of a representative experiment is shown. Phospho-Akt/total Akt at time zero is set as 1. Note that ISO-induced activation of Akt was observed even at 0.1 μM. The result is representative of three experiments.
B) Neonatal rat cardiac myocytes were stimulated with ISO with prazosin. C) Neonatal rat cardiac myocytes were stimulated with indicated concentrations of ISO for the indicated durations. Levels of phospho-Tyr 416 Src and total Src were determined by immunoblotting and densitometric analyses. Note that ISO-induced activation of Src was easily observed at 1 μM. The result is representative of three experiments.

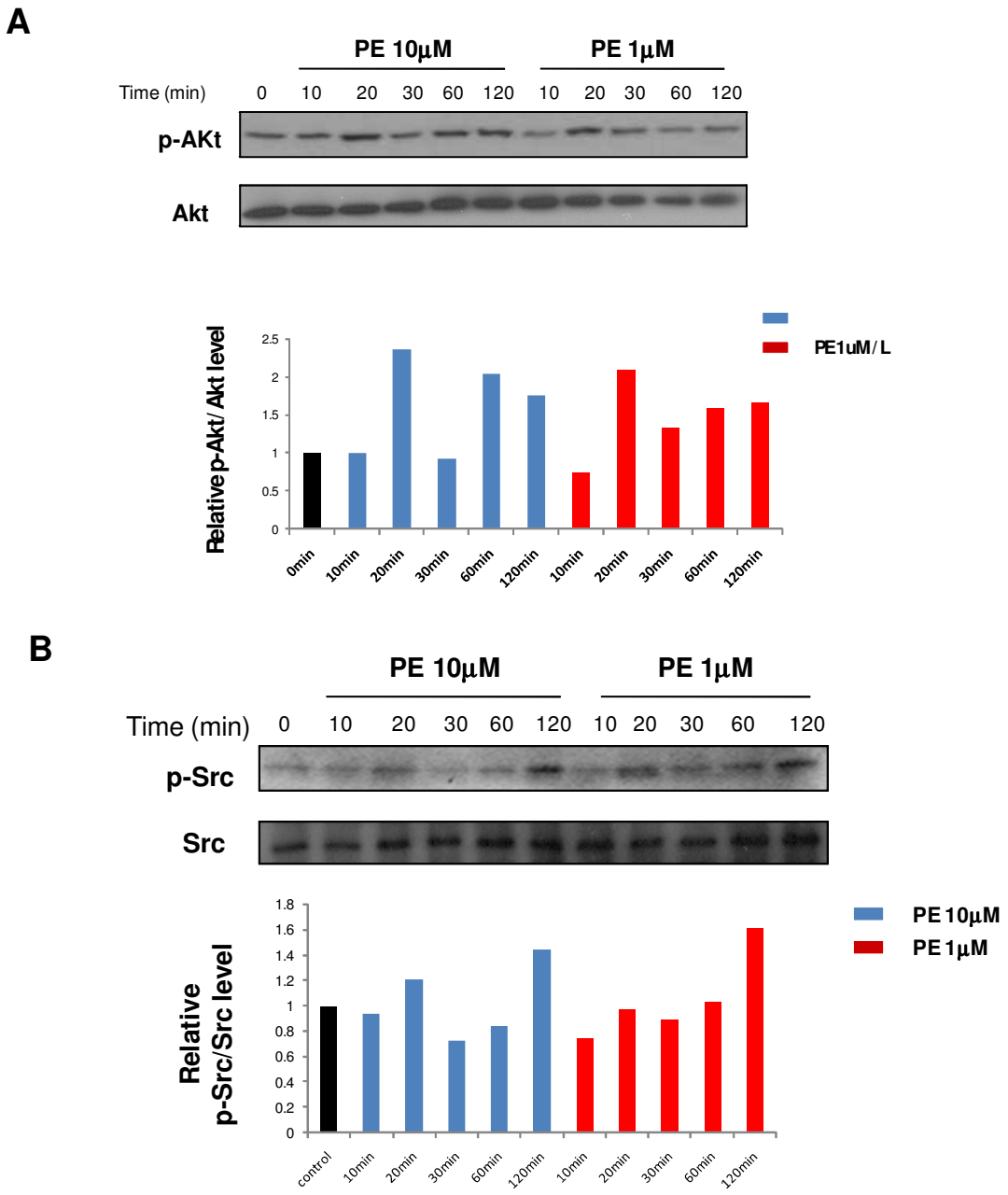

Figure II Neonatal rat cardiac myocytes were stimulated with indicated concentrations of phenylephrine (PE) for the indicated durations. A) Levels of phospho-Ser 473 Akt and total Akt were determined by immunoblotting and densitometric analyses. In the lower panel a bar graph of relative phospho-Akt/total Akt of a representative experiment is shown. Phospho-Akt/total Akt at time zero is set as 1. Note that activation of Akt by PE was modest compared with that by isoproterenol. The result is representative of three experiments. B) Levels of phospho-Tyr 416 Src and total Src were determined by immunoblotting and densitometric analyses. In the lower panel a bar graph of relative phospho-Src/total Src of a representative experiment is shown. Phospho-Src/total Src at time zero is set as 1. Note that activation of Src by PE at early phase was modest compared with that by isoproterenol. The result is representative of three experiments.

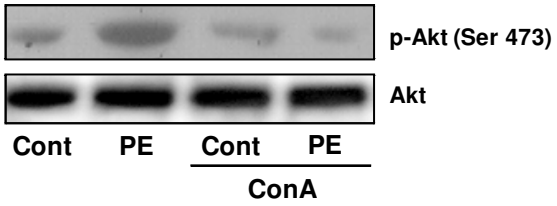

Figure III Neonatal rat cardiac myocytes were pretreated with concanavlin A (Con A) for 30 min and then stimulated with phenylephrine (PE, 10 μM). Activation of Akt was evaluated by immunoblotting with anti-phospho-Akt and anti-total Akt antibodies. Note that PE-induced activation of Akt was inhibited in the presence of Con A.