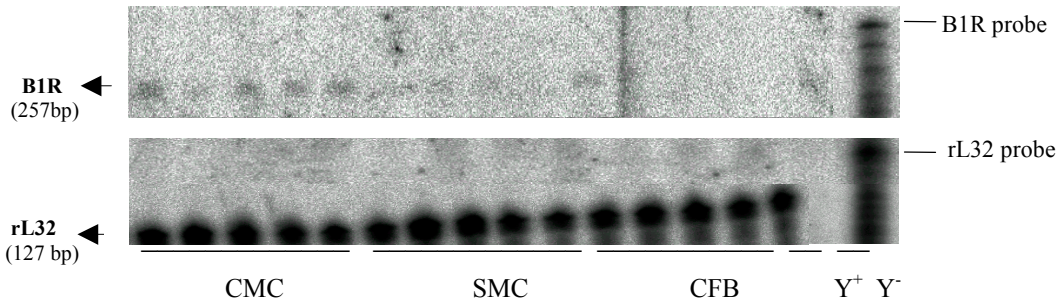
A



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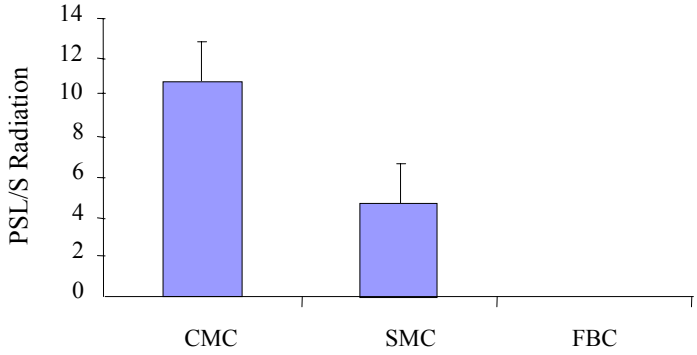
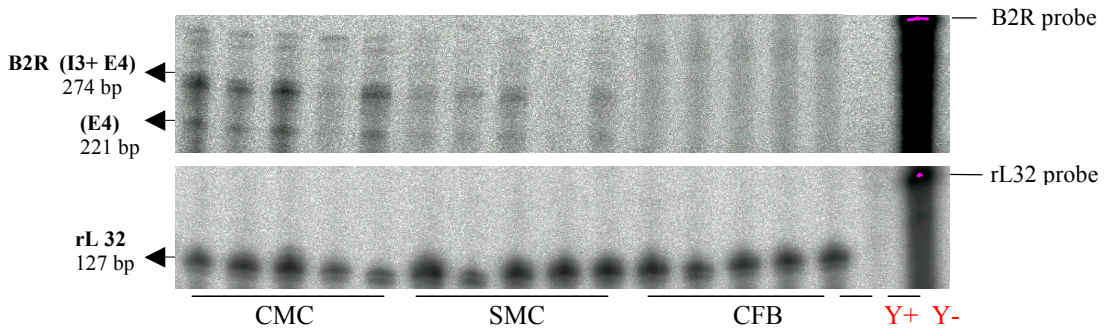


Fig 3. 1 Detection of B1R mRNA in rat CMC, CFB, and SMC. Confluent cells were treated with serum free medium for 12 h, total RNA was isolated and the amount of B1R mRNA was analysed by RPA as described in methods. The amount of B1R mRNA was compared to the amount of rL-32 mRNA. A) Representative RPA assay analysis (n=5, each group) showing B1 mRNA (257 bp) versus rL32 mRNA (127 bp) expression in different cell types (CMC, CFB, and SMC). B) Quantitation of B1R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels. In this and succeeding figures, bar graph represents the means ± SE of the intensities of the bands from RPA blots. PSL/S, photostimulated luminescence per area.

A



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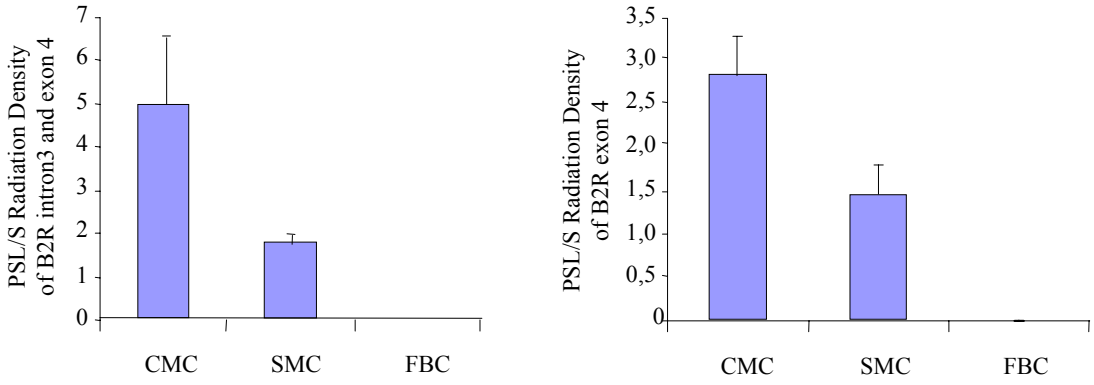


Fig 3. 2 Detection of B2R mRNA in rat CMC, CFB, and SMC. Confluent cells were treated with serum free medium for 12 h, total RNA was isolated and the levels of B2R mRNA was analysed by RPA. The amount of B2R mRNA was compared to the amount of rL32 mRNA. A) Representative RPA assay analysis (n=5, each group) showing B2 mRNA (intron3+exon4 [I3+E4], 274 bp; exon4 [E4], 221 bp) versus rL32 mRNA (127 bp) expression in different cell types (CMC, CFB, and SMC). B) Quantitation of B2R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.

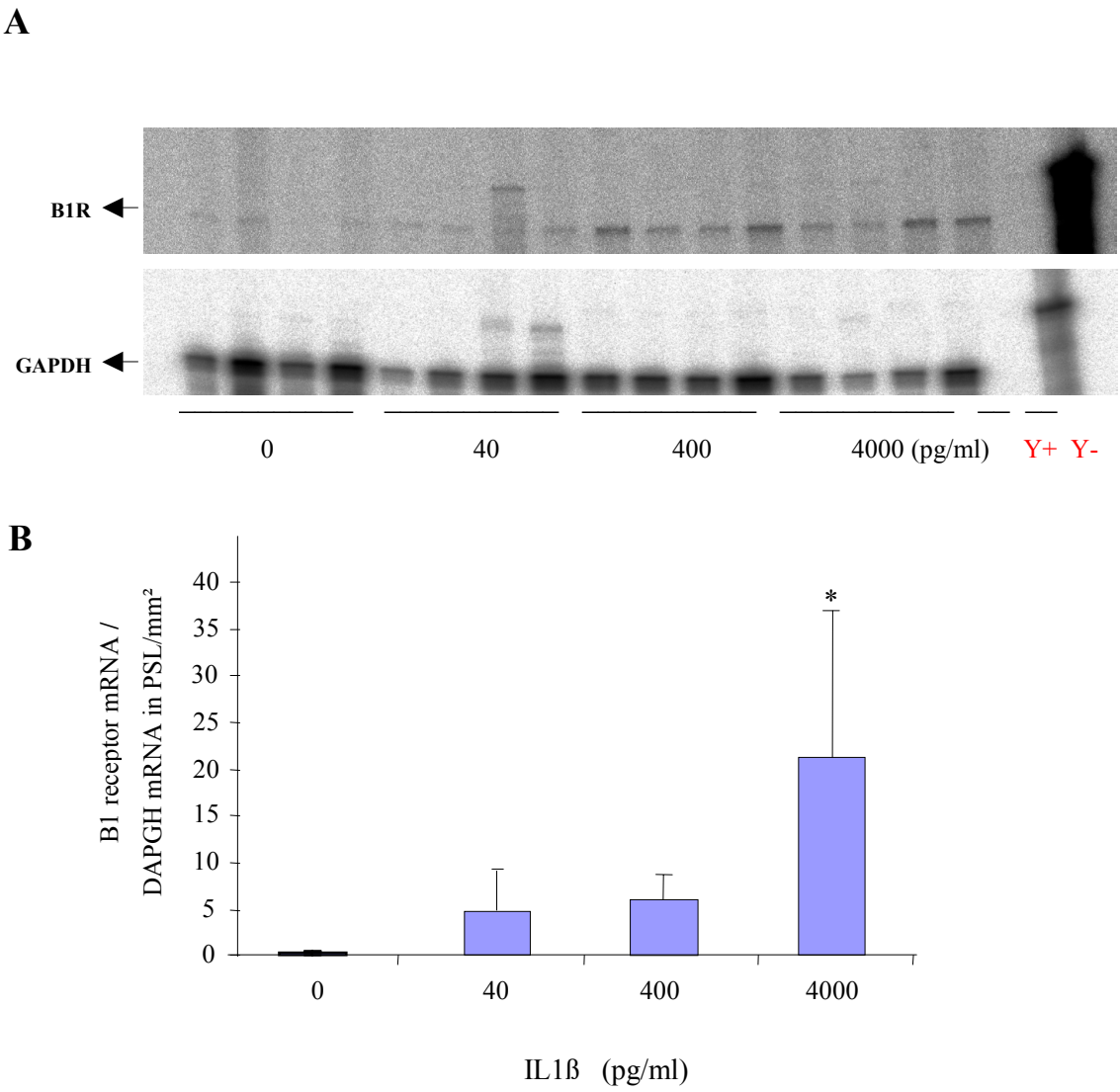


*Fig 3. 3 B1R mRNA expression after 12 h IL1ß treatment in rat CMC. Beating CMC were pretreated with serum free medium for 12 h, then treated with IL1ß using different concentrations for another 12 h. Total RNA was extracted and the amount of B1R mRNA was analysed by RPA as described in methods. A) Autoradiograph of RPA blot (n=4, each group) showing B1R mRNA versus GAPDH mRNA expression. B) Quantitation of B1R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels. *P<0.05 versus 0 pg/ml. (P<0.05 is considered significant)*

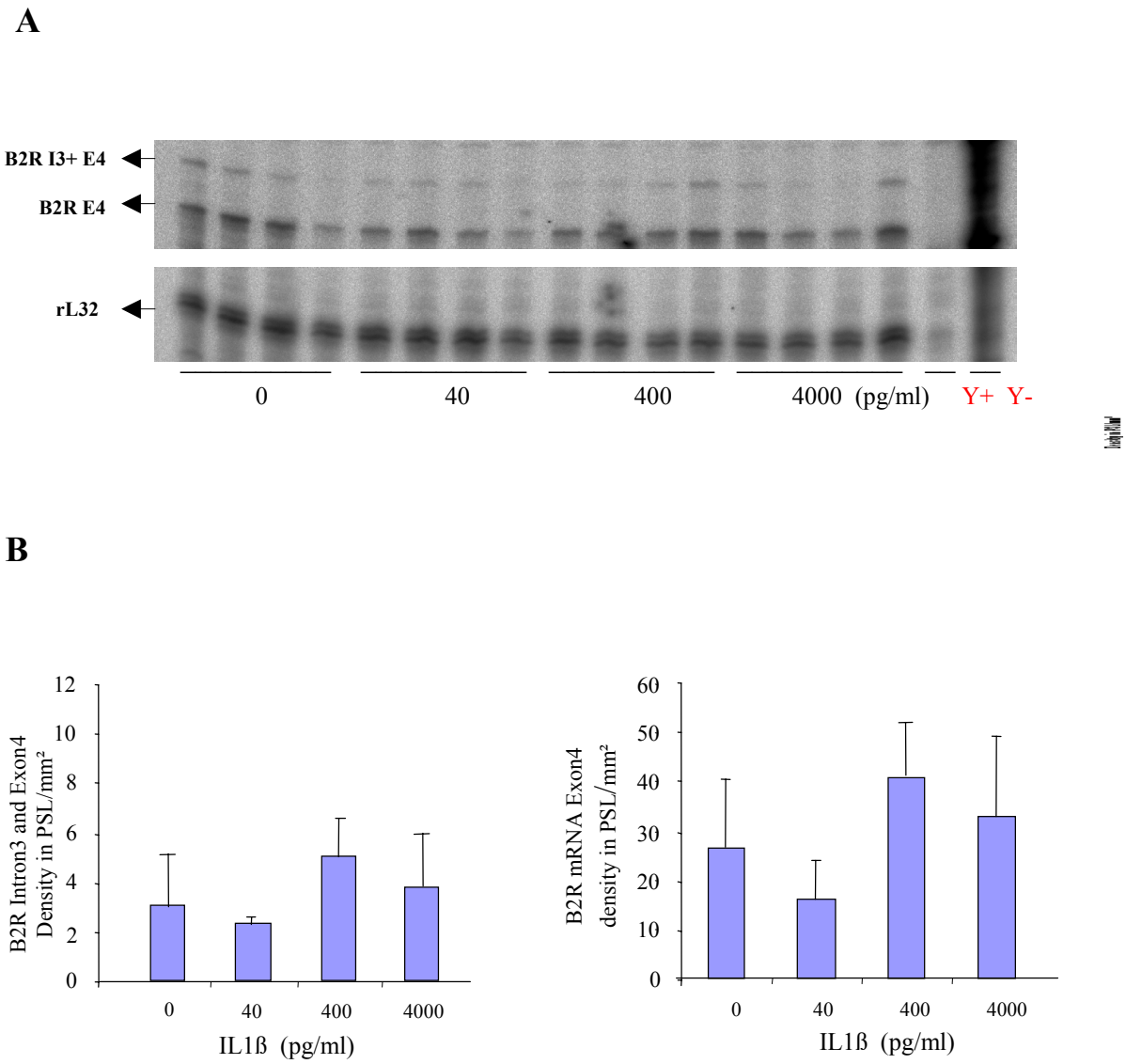


Fig 3. 4 Expression of B2R mRNA after 12 h IL1ß treatment in rat CMC. Beating CMC were pretreated with serum free medium for 12 h, followed by another 12 h treatment with IL1ß at different concentrations (0, 40, 400, and 4000 pg/ml). Total RNA was extracted and the amount of B2R mRNA was analysed by RPA as described in methods. A) Autoradiographs of RPA blots (n=4, each group) showing B2R mRNA versus rL32 mRNA. B) Quantitation of B2R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.

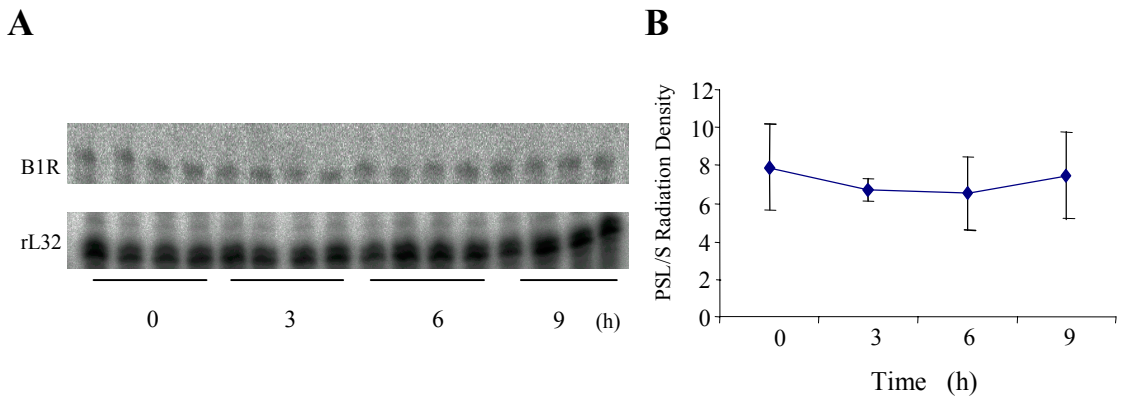


Fig 3. 5 Time course of B1R mRNA levels relative to rL32 mRNA levels in CMC after cultivating these cells in serum free medium for varying times. A) Representative RPA assay analysis (n=4, each group) showing B1R mRNA versus rL32 mRNA expression. B) Quantitation of B1R expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.

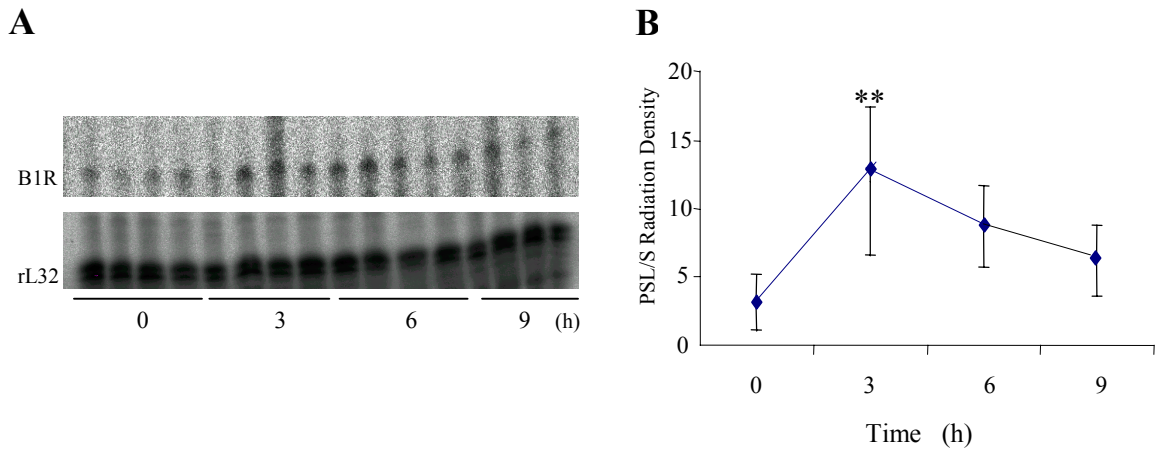


*Fig 3. 6 Time course of B1R mRNA levels relative to rL32 mRNA levels in CMC in response to 400 pg/ml IL1ß for varying times. A) Representative RPA assay analysis (n=4, each group) showing B1R mRNA versus rL32 mRNA expression. B) Quantitation of B1R expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels. ** P<0.01 versus 0 h*

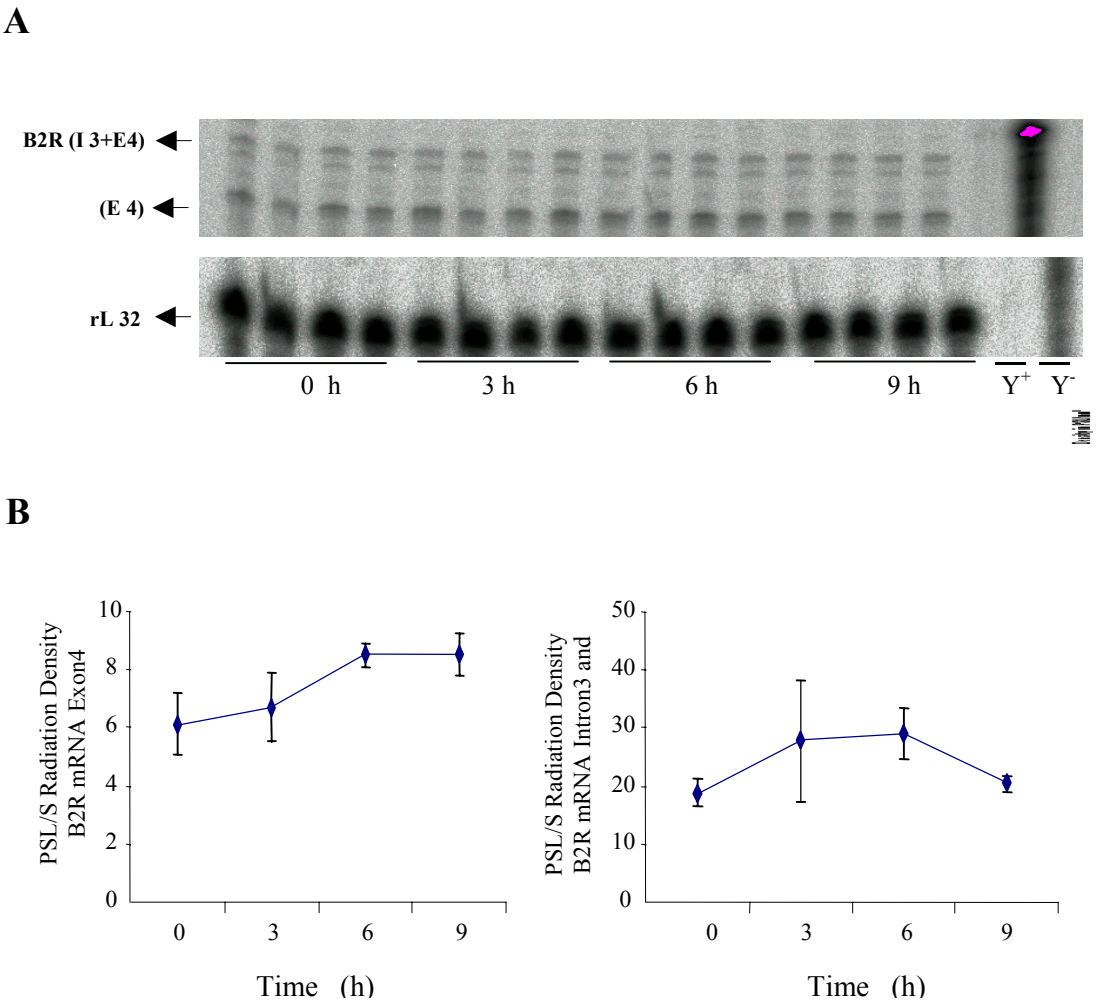


Fig 3. 7 Time course of B2R mRNA levels relative to mRNA levels of rL32 in CMC after cultivating these cells in serum free medium for varying times. A) Representative RPA assay analysis (n=4, each group) showing B2R mRNA versus rL32 mRNA expression. B) Quantitation of B2R. Expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.

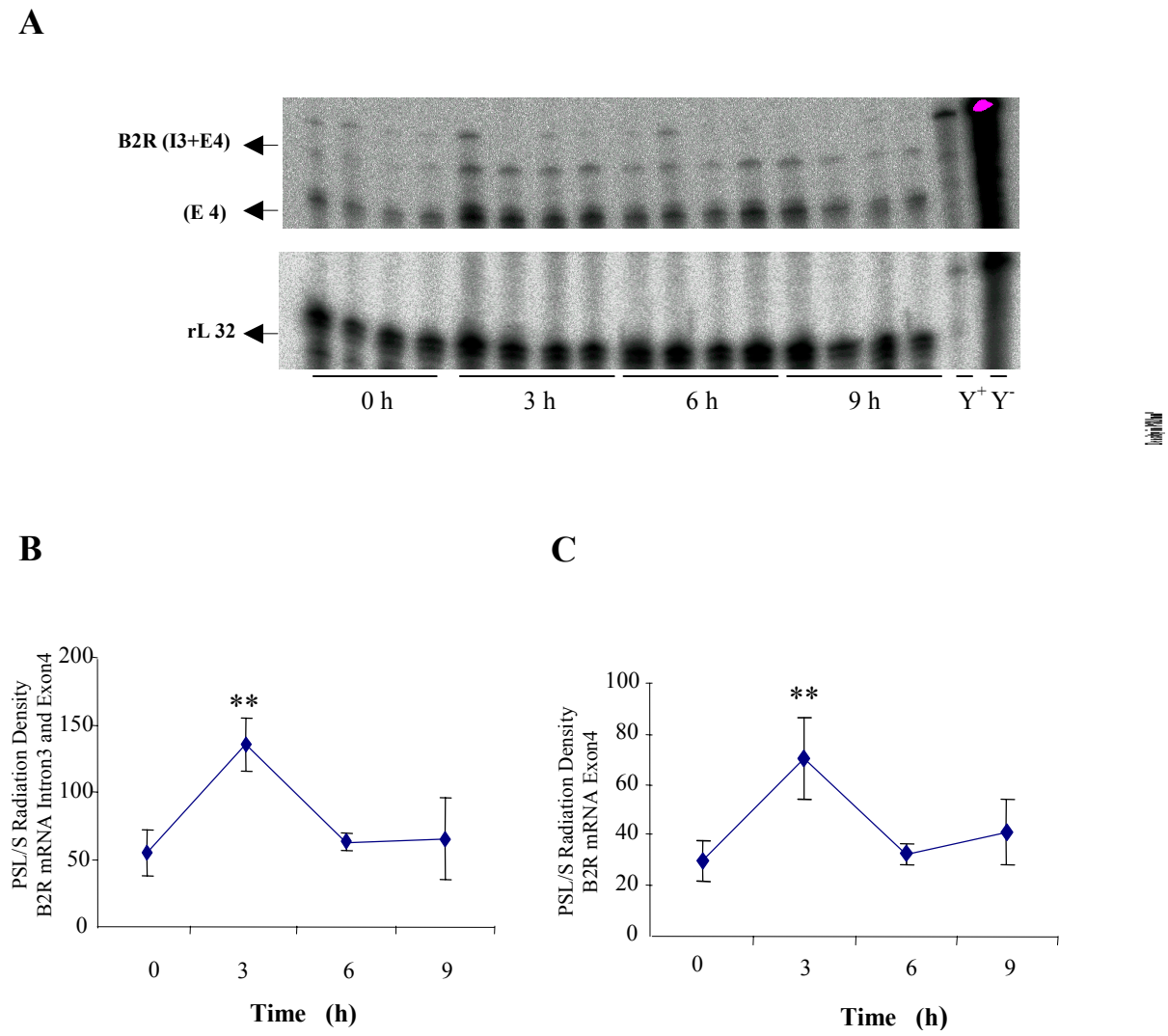


*Fig 3. 8 mRNA levels of B2R relative to mRNA levels of rL32 in CMC in response to 400 pg/ml IL1ß for varying times. A) Autoradiographs of RPA blots (n=4, each group) showing B2R versus rL32 expression after exposure to IL1ß (400 pg/ml) for varying times. B) Quantitation of B2R mRNA intron 3 and exon 4 and C) B2R mRNA exon 4 expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels (**P<0.01 versus 0 h).*

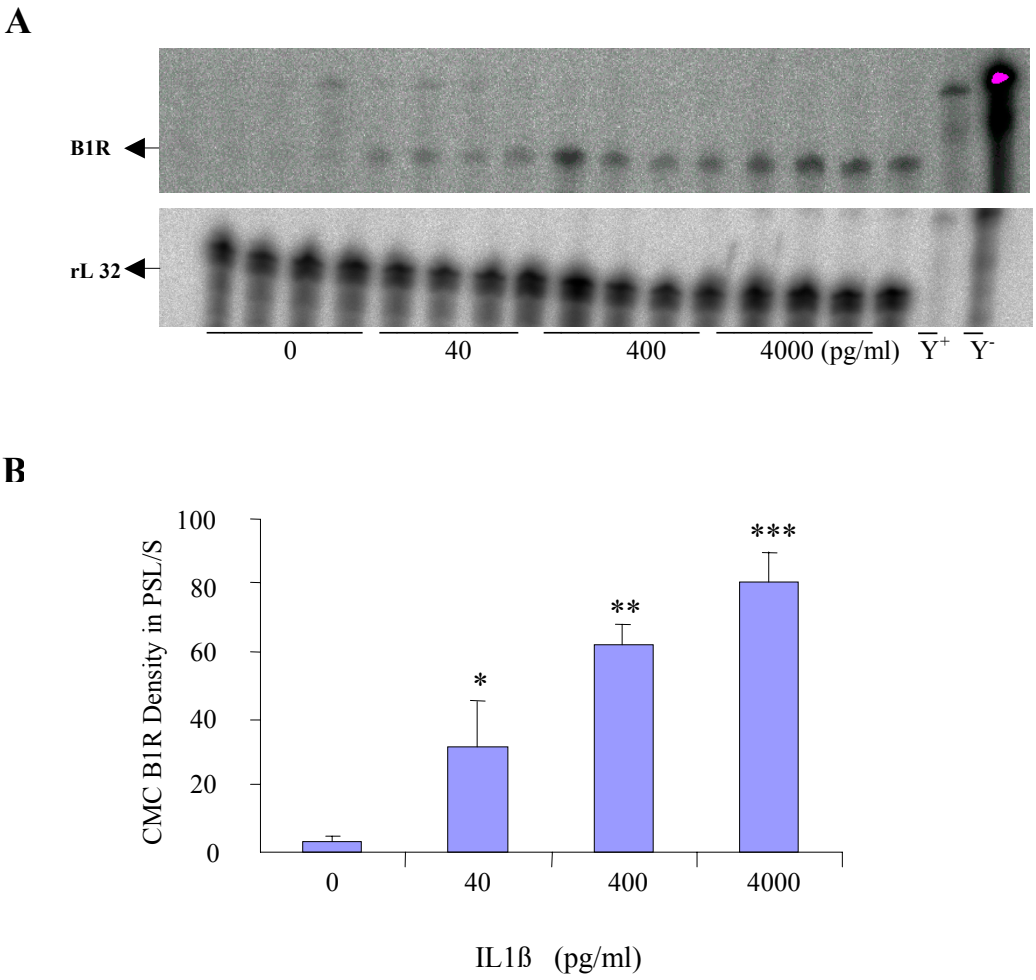
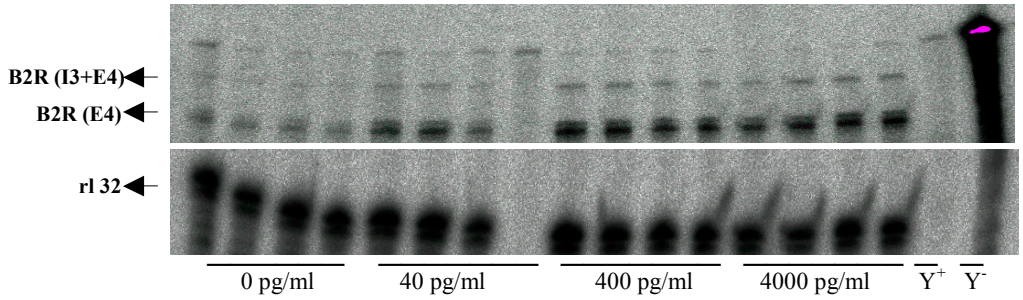


*Fig 3. 9 Expression of B1R mRNA after 3 h IL1ß treatment in rat CMC. Beating CMC were pretreated with serum free medium for 12 h, followed by another 3 h exposure to IL1ß at different concentrations (0, 40, 400, and 4000 pg/ml). Total RNA was extracted and the amount of B1R mRNA was analysed by RPA as described in methods. A) Autoradiographs of RPA blots showing B1R mRNA versus rL32 mRNA expression after exposure to IL1ß at different concentrations. B) Quantitation of B1R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels (*P <0.05,**P<0.01,***P<0.005 versus 0 pg/ml).*

A



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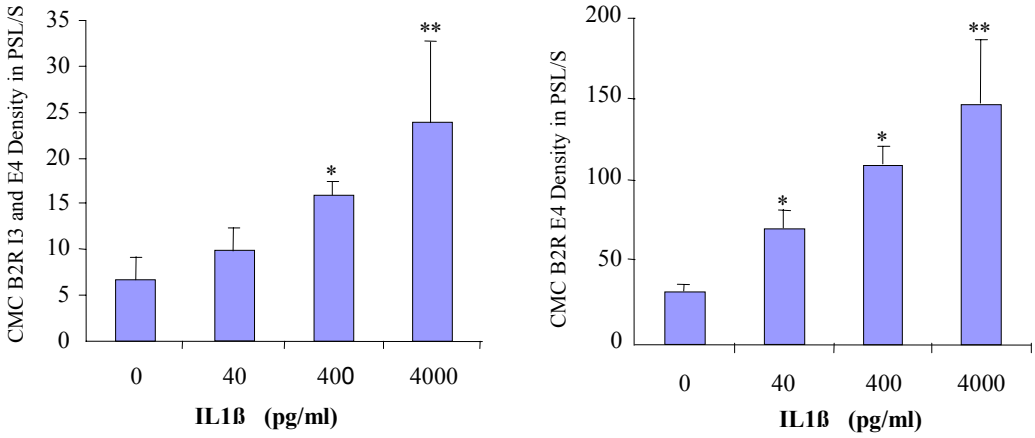


Fig 3. 10 Expression of B2R mRNA after 3h IL1ß treatment in rat CMC. Beating CMC were pretreated with serum free medium for 12 h, followed by another 3 h exposure to IL1ß at different concentrations (0, 40, 400, and 4000 pg/ml). Total RNA was extracted and the amount of B2R mRNA was analysed by RPA as described in methods. A) Autoradiographs of RPA blots showing B2R mRNA versus rL32 mRNA expression after exposure to IL1ß. B) Quantitation of B2R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.

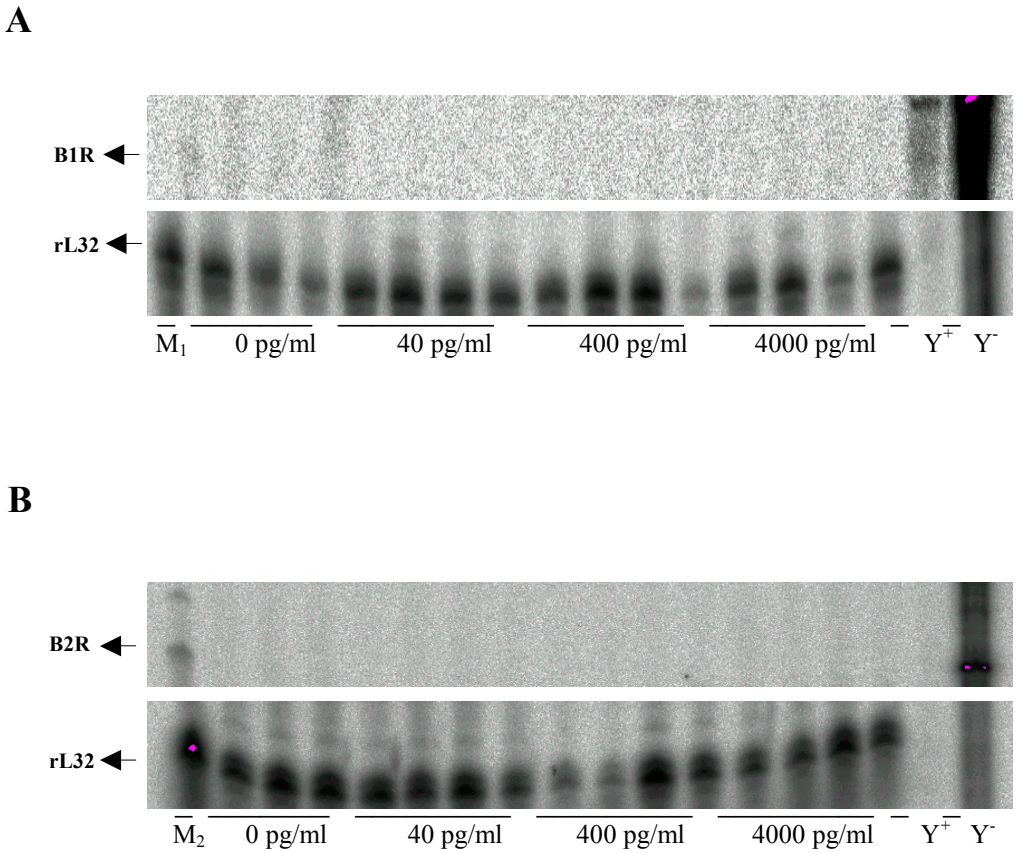
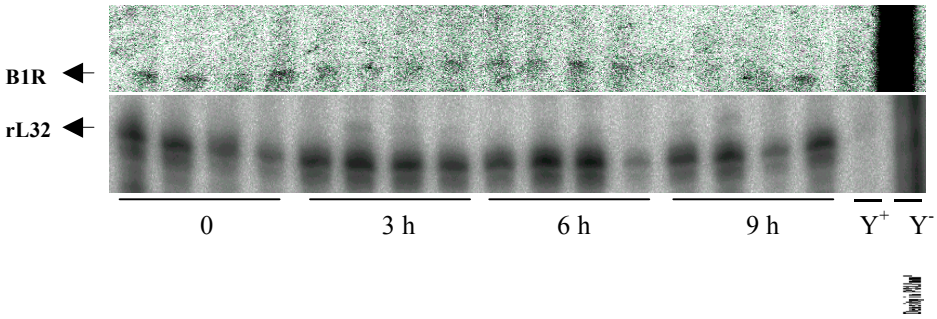


Fig 3. 11 Detection of bradykinin B1 and B2 receptors in CFB. Confluent cells were treated with serum free medium for 12 h, followed by another 3 h exposure to IL1ß at different concentrations (0, 40, 400, and 4000 pg/ml), then total RNA was prepared and both B1R and B2R mRNA were detected by RPA as described in methods, Using this method, no B1R (A) or B2R (B) mRNA bands were detected in CFB. The B1R or B2R mRNA signals (M_1, M_2) detected in the gel shows that the lack of interest bands was not due to methodological problems. M_1/M_2, RNA were isolated from rat myocardium and ileum respectively.

A



B

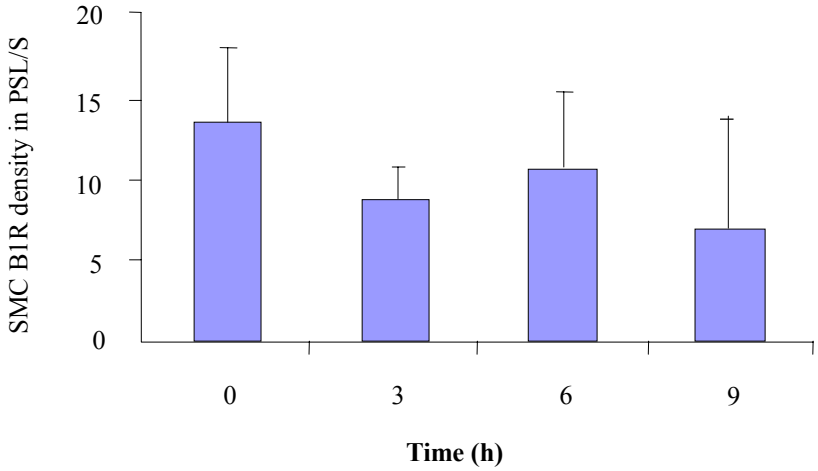
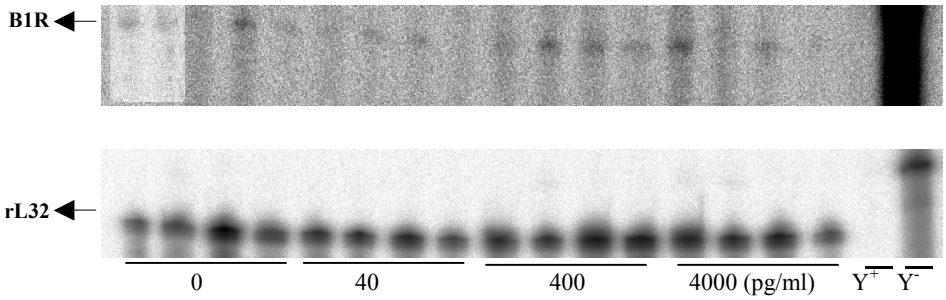


Fig 3. 12 Expression of B1R mRNA after 400 pg/ml IL1ß treatment in rat smooth muscle cells. Confluent cells were pretreated with serum free medium for 12 h, followed by exposure to 400 pg/ml IL1ß for varying times. A) Autoradiographs of RPA blots (n=4, each group) showing B1R versus rL32 expression. B) Quantitation of B1R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.

A



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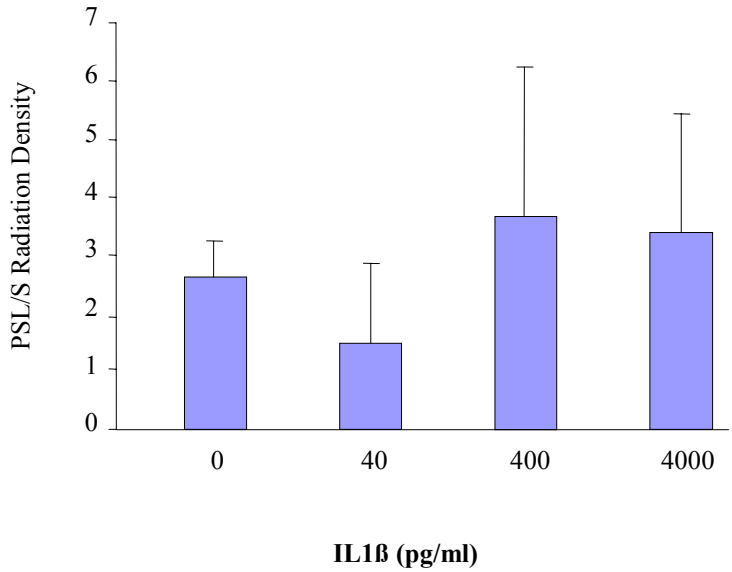
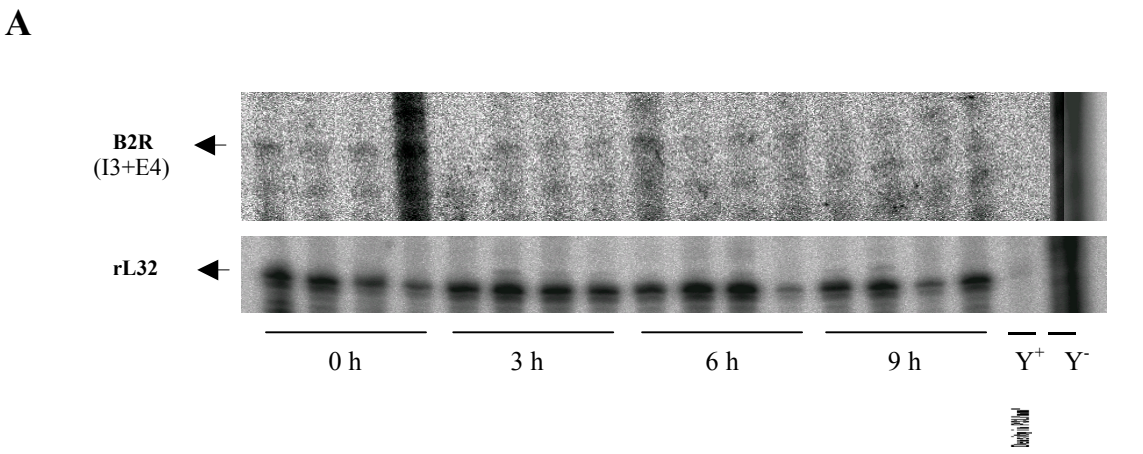


Fig 3. 13 Expression of B1R mRNA after 3h IL1ß treatment in rat SMC. Confluent cells were pretreated with serum free medium for 12 h, followed by another 3 h exposure to IL1ß at different concentrations (0, 40, 400, and 4000 pg/ml). Total RNA was extracted and the amount of B1R mRNA was analysed by RPA as described in methods. A) Polyacrylamide gel with protected hybrids for B1R mRNA and rL32 mRNA. B) Quantitation of B1R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.



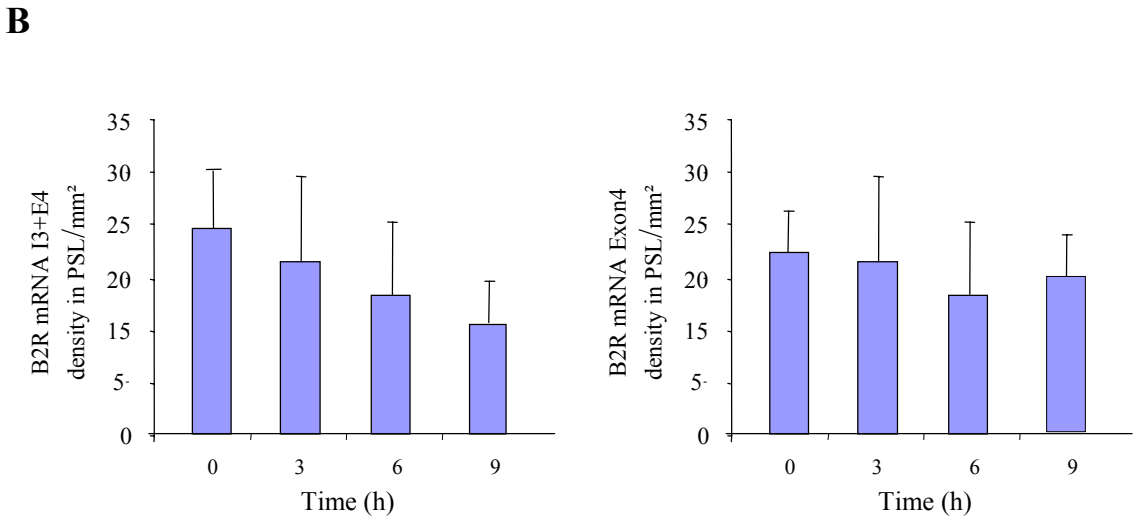
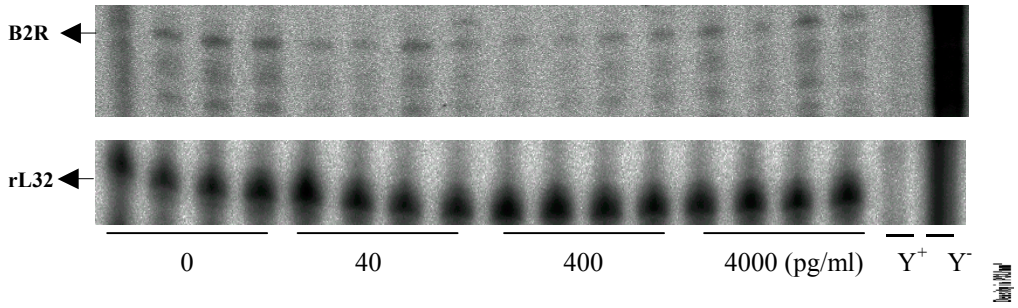


Fig 3. 14 Expression of B2R mRNA after 400 pg/ml IL1ß treatment in rat smooth muscle cells. Confluent cells were pretreated with serum free medium for 12 h, followed by exposure to 400 pg/ml IL1ß for varying times. A) Autoradiographs of RPA blots (n=4, each group) showing B2R versus rL32 expression. B) Quantitation of B2R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.

A



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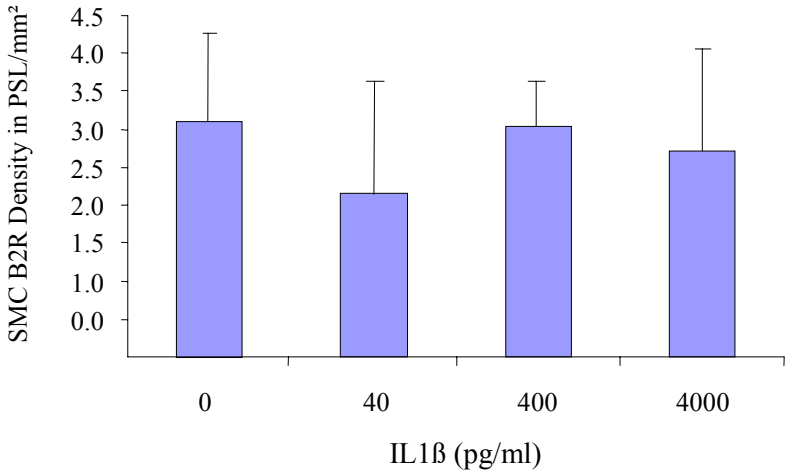
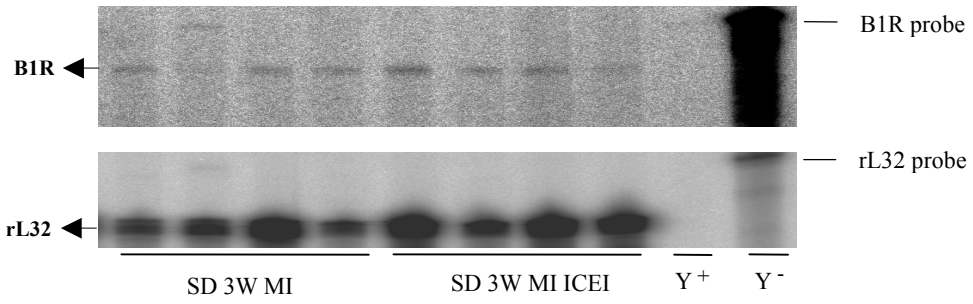


Fig 3. 15 Expression of B2R mRNA after 3h IL1ß treatment in rat SMC. Confluent cells were pretreated with serum free medium for 12 h, followed by another 3 h exposure to IL1ß at different concentrations (0, 40, 400, and 4000 pg/ml). A) Representative RPA assay analysis (n=4, each group) showing B2 mRNA versus rL32 mRNA expression. B) Quantitation of B2R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.

A



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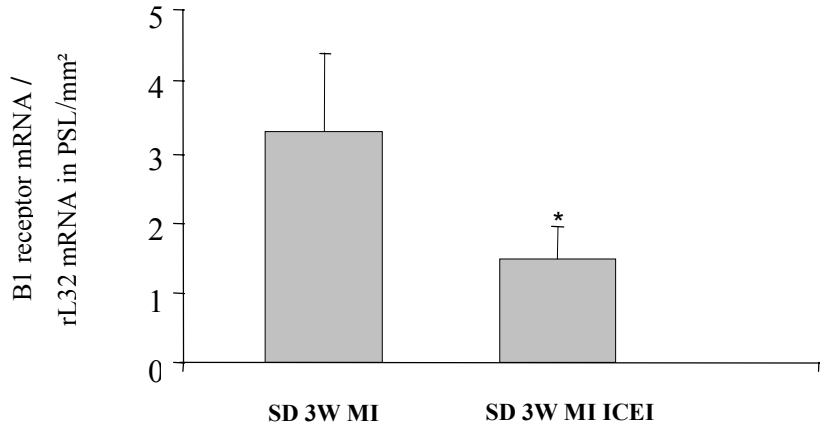
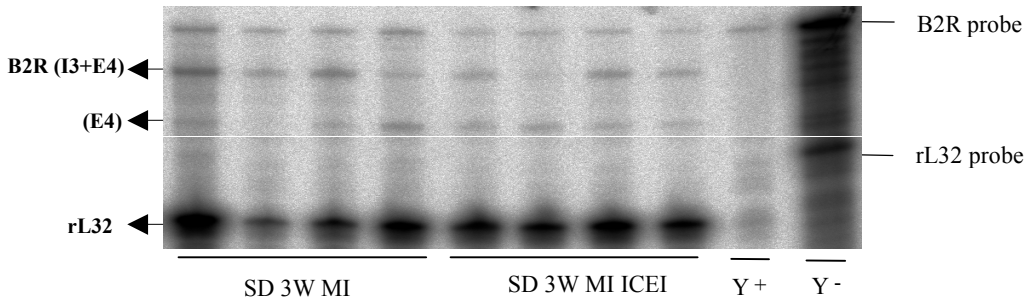


*Fig 3. 16 B1R mRNA expression in the left ventricle (infarcted area) 3 weeks after induction of MI with or without treatment by ICEI. A) Autoradiographs of RPA blots showing B1R mRNA versus rL32 mRNA expression. B) Quantitation of B1R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels (*P<0.05 versus without ICEI treatment).*

A



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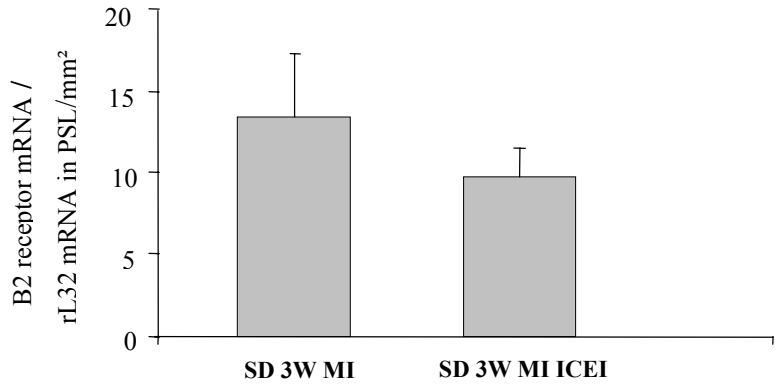

Fig 3. 17 B2R mRNA expression in the left ventricle (infarcted area) 3 weeks after induction of MI with or without treatment by ICEI. A) Autoradiographs of RPA blots showing B2R mRNA versus rL32 mRNA expression. B) Quantitation of B2R mRNA expression after autoradiographic signal analysis. Data are shown as multiples after normalisation to rL32 mRNA levels.