

## ROLE OF PROTEIN KINASE C AND RHO KINASE IN THE RAT-SELECTIVE VASOCONSTRICTOR EFFECT OF NORMORMIDE

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Norbormide (NRB) is a synthetic selective toxin for the rat in which it induces an intense and irreversible vasoconstriction that leads to heart failure and death. Even at very high doses, NRB failed to induce vasoconstriction in non rat species, therefore it can be considered a rat-specific vasoconstrictor agent. The mechanism underlying NRB-induced vasoconstriction has been only partially elucidated and seems to involve activation of a PLC-PCK pathway causing  $Ca^{2+}$  entry through store-operated channels; however, the receptor involved in NRB action remains unknown. PKC and Rho kinase (ROK) are downstream effectors activated during agonist-induced vascular smooth muscle contraction. In this study, we investigated the relative roles of PKC and ROK in NRB-induced vasoconstriction. To this goal, using the rat caudal artery as a model, we measured NRB-induced contraction in the presence or absence of PKC and ROK inhibitors. Moreover, PKC and ROK activation were evaluated by western blot analysis. In endothelium-free rat caudal artery rings NRB (0.1 - 56  $\mu$ M) induced a concentration-dependent vasoconstriction which could be inhibited dose-dependently by the selective PKC inhibitor GF109203X (1 - 10  $\mu$ M). Same results were obtained using the ROK inhibitors HA1077 and Y21632 (1 - 10  $\mu$ M) and the MLC kinase inhibitors ML-7 and ML-9 (10 - 30  $\mu$ M). Exposure of rat caudal arteries to NRB (5  $\mu$ M) for 3 and 10 min induced a time-dependent increase in both PKC and ROK. Among the PKC isoforms described in vascular smooth muscle, only the  $Ca^{2+}$ -dependent ones were activated by NRB. In conclusion, in this study we show that NRB-induced vasoconstriction, similarly to the vasoconstriction induced by most receptor-coupled agonists, is mediated by both PKC and ROK activation. On the basis of these results we hypothesise that NRB could act on a receptor selectively expressed/active on the rat vascular myocytes.