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Lack of an Effect of Soman on Norepinephrine–Induced Relaxations of Porcine Coronary Arteries

Chao-Yu Hu and Casey P. Robinson

College of Pharmacy, University of Oklahoma, Health Sciences Center, Oklahoma City, Oklahoma 73190, USA

Soman (pinacolyl methylphosphonofluoridate) is an organophosphonate cholinesterase (ChE) inhibitor. It can alter blood pressure (Preston and Heath, 1972 a, b; Brezenoff *et al.* 1984). It is not used commercially, but the potential exists for environmental contamination during storage or disposal. Part of the blood pressure changes may be due to effects on blood vessels as soman alters the contractility of isolated arteries (Hu *et al.* 1988; Hu and Robinson 1988a). Effects on norepinephrine (NE) metabolism, release, and uptake following seven-day soman administration have also been reported (Hu and Robinson 1988b). Because soman alters NE handling at the neuroeffector system in systemic vasculature (at predominantly *alpha*-adrenergic receptors), it was thought of interest to study soman's effects on NE-induced relaxations of coronary arteries (at primarily *beta*-adrenergic receptors). Norepinephrine contracts most isolated blood vessels, but relaxes coronary arteries. Contractions of most blood vessels to NE result from stimulating primarily *alpha*-1 adrenergic receptors, while coronary arteries relax to NE by *beta*-receptor stimulation (Parrat and Wadsworth 1970; Baron *et al.* 1972).

This study was designed to determine whether or not soman alters the resting tension or the NE-induced relaxation of porcine coronary arteries. A high concentration of soman was used. Had effects been observed, lower concentrations would also have been used.

MATERIALS AND METHODS

Hearts from domestic, approximately 115 kg, feeder pigs obtained from a local slaughterhouse were placed immediately into ice-cold Krebs-Henseleit solution. The Krebs-Henseleit solution contained NaCl, 119 mM; KCl, 4.8 mM; $CaCl_2$, 1.6 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 24.9 mM; dextrose, 11.1 mM; and ascorbic acid, 0.057 mM in glass-distilled water and was aerated with 95% O_2 - 5% CO_2 for 30 min prior to use. Coronary arteries and portions of their branches were removed, carefully cleaned of connective tissue, spirally cut, and stored at 4°C. Care was taken to not damage the

Send reprint requests to Casey Robinson at the above address.

endothelium. None were used on the day they were removed; all were used either 24 or 48 hours after removal. The four sizes of arteries used included the following: very large (>4.0 mm o.d.), large (1.5 to 2.5 mm o.d.), medium (0.8 to 1.2 mm o.d.), and small (<0.5 mm o.d.). Each coronary artery strip was tied at both ends and attached to a stainless steel rod at the bottom and a microdisplacement myograph transducer F-60 (Narco Biosystems) at the top. The strips were suspended in separate water-jacketed organ baths maintained at 38°C. Isometric tension changes were recorded on an MK-IV Physiograph (Narco Biosystems). The artery strips were allowed to equilibrate for approximately 2 hr under the following tensions: very large, 2.0 g; large, 1.5 g; medium, 1.0 g; and small 0.5 g.

After equilibration the muscle strips were contracted with 30 mM KCl (large and very large) or 60 mM KCl (small and medium coronary arteries). The higher KCl concentration was used to contract the smaller arteries because they did not contract much to 30 mM KCl. These contractions generally reached a plateau within 20 min. Norepinephrine (0.01 - 100 μM) was added cumulatively, with each dose added after responses to the previous dose were maximal, and followed by the addition of 100 μM papaverine to relax the strips completely. Papaverine-induced relaxations were taken as 100% relaxation. Strips were washed three times immediately after maximal relaxation and approximately every 15 min during a 90 min waiting period. Strips were then again contracted with KCl, then 10 μM soman was added to half of the strips of each size. Those strips not receiving soman served as controls for changes in the first and second responses to NE. Ten min after soman addition NE was added cumulatively, again followed by papaverine.

Tension changes to NE before and after exposure to soman were corrected for changes in responses of the control strips and compared using a two-way analysis of variance. The effects of soman on KCl-induced contractions were detemined when given before the KCl was added and also when given after the contraction to KCl had plateaued. Differences were compared by the Student's t test with $p < 0.05$ considered significant.

Soman was obtained from the United States Army Medical Research Institute of Chemical Defense, Aberbeen Proving Ground, MD.

RESULTS AND DISCUSSION

All four sizes of coronary artery segments were contracted by KCl, relaxed by NE, and further relaxed to baseline or below by 100 μM papaverine.

Prior exposure of the strips to 10 μM soman did not alter the NE-induced relaxation in any size coronary artery segment (Fig. 1) in the studies on the cumulative addition of NE.

Soman did not alter the resting tension of coronary artery strips, or the tension of potassium-contracted strips. A tracing shows the contractile

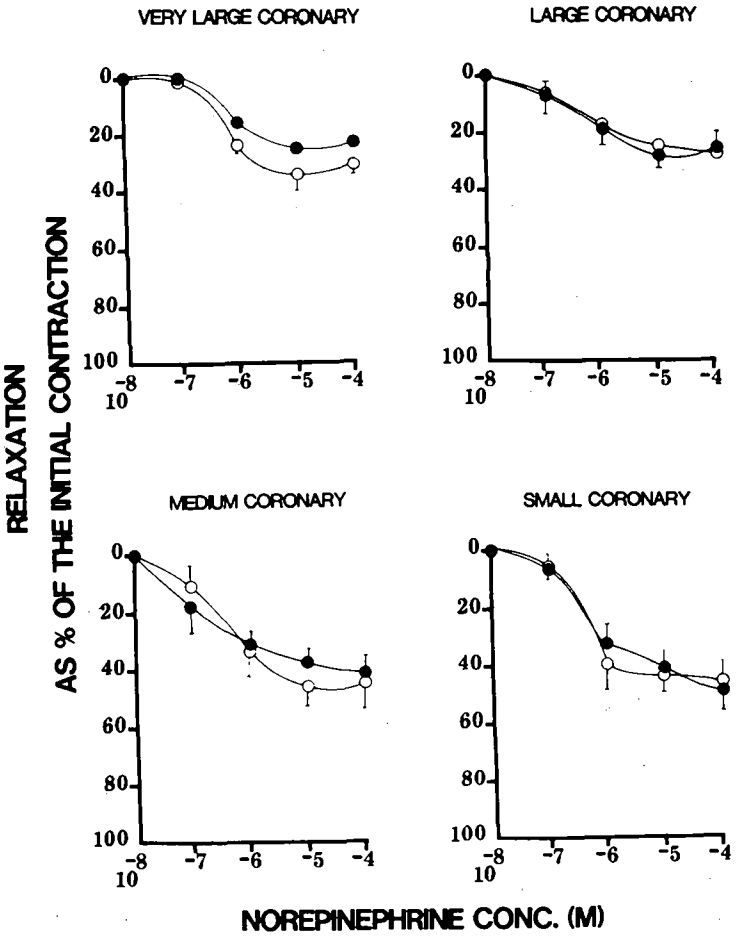

Figure 1. The effects of 10 μM on porcine coronary arteries contracted by KCl on relaxations to cumulatively added norepinephrine. Each point is the mean S.E. of observations from 4-9 coronary arteries.

○ = control
● = soman treated

tension on addition of 10 μM soman to a strip before being contracted with potassium (Fig. 2A) and to another strip already contracted with potassium (Fig. 2B). No changes in tension to soman were observed.

Porcine coronary arteries which have been contracted by potassium, prostaglandin $F_{2\alpha}$, or other agonists are relaxed by NE (Parratt and Wadsworth 1970; Baron *et al.* 1972; Horst and Robinson 1984, 1985). This results from stimulating *beta*-adrenergic receptors, rather than *alpha*-

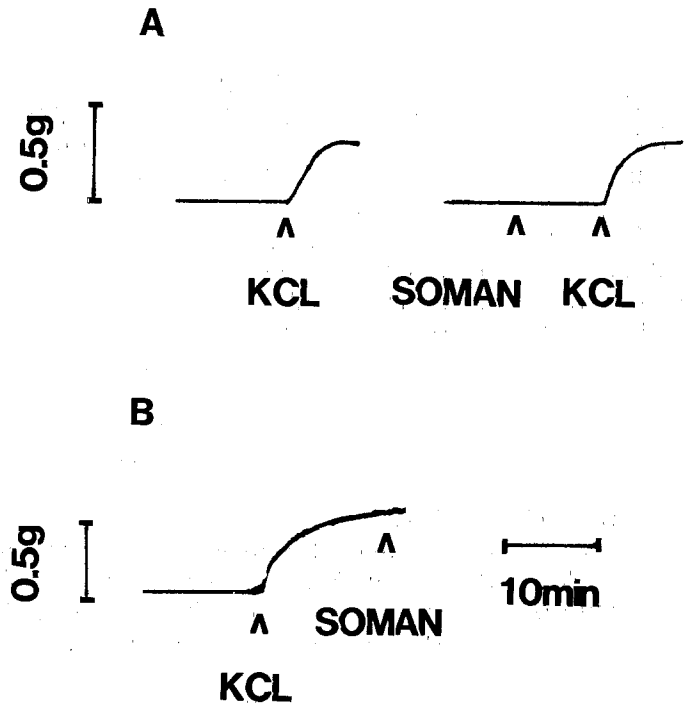

Figure 2 A. Contraction of a medium-sized coronary artery strip to 60mM KCl before (left) and after (right) 10 μM soman.
B. Effect of 10 μM soman on a KCl-contracted medium-sized coronary artery strip.

adrenergic receptors which mediate vascular contractions. The lack of effect of soman on coronary arteries may relate to differences in actions at the *alpha* and *beta* adrenergic receptors.

In summary, neither antagonism of tonic NE-induced relaxation nor direct contractile activity by soman are likely to contribute to soman-induced toxicity.

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