proton and a vinyl proton signal at 5.65 (br d, 1 H, $J=6$ Hz).

The sulfate **3** had the molecular formula $C_{28}H_{43}O_6SNa$. The EI mass-spectrum contained a peak at m/z 410 with fragmentation peaks at m/z 366. 285 and 241, indicating the presence of a C_9H_{17} side chain. The ^1H NMR-spectrum contained 4 methyl signals at δ 0.66 (s, 3 H), 0.94 (d, 3 H, $J=7$ Hz) and 1.02 (d, 6 H, $J=7$ Hz). The methyl signal at δ 1.02 was coupled to a vinyl proton signal at 2.23 (septet, 1 H, $J=7$ Hz, C-25). The methylene group attached at C-24 gave rise to signals at 4.65 (br s, 1 H) and 4.70 (br s, 1 H), indicating the presence of the 24-methylene cholesterol carbon skeleton. The ^{13}C NMR-spectrum contained signals at δ 21.5 (q), 21.7 (q), 32.9 (s), 106.3 (t) and 155.6 (s) for carbons 24–28 in the 24-methylene sterol side chain. The remaining spectral data were all consistent with the proposed structure for sulfate **3**.

During the hydrolysis of the sulfate **3**, the double bond in the side chain migrated to give lactone **9** and ester **10**, both of which have a tetrasubstituted $^{24}\Delta$-olefinic bond. The lactone **9**, $[\alpha]_D$ $-96°$ (c=0.45, $CHCl_3$), had the molecular formula $C_{28}H_{42}O_2$. The ^1H NMR-spectrum contained 5 methyl signals at δ 0.85 (s, 3 H), 0.96 (d, 3 H, $J=7$ Hz), 1.57 (s, 6 H) and 1.62 (s, 3 H) together with signals at 4.70 (m, 1 H, C-3) and 2.62 (m, 1 H, C-6). The methyl ester **10** could not be obtained completely pure. However, the EI mass-spectrum contained a peak at m/z 442 corresponding to the molecular formula $C_{29}H_{46}O_3$. The ^1H NMR-spectrum contained 5 methyl signals at δ 0.61 (s, 3 H), 0.98 (d, 3 H, $J=7$ Hz), 1.60 (s, 3 H) and 1.62 (s, 6 H), a methoxy signal at 3.71 (s, 1 H) and signals at 3.54 (m, 1 H, $J=11$, 11, 4, 4 Hz) and 5.65 (br d, 1 H, $J=4$ Hz).

The hydrolysis of each of the sulfates **1–3** gave a mixture of products from which we isolated and characterized the major products. Among the minor products from each hydrolysis were the corresponding *i*-sterols, as indicated by the presence of cyclopropyl proton signals in the ^1H NMR-spectrum, but none of these were fully characterized.

The biological activity of the sterol sulfates presents a rather complicated picture. Each of the pure sterol sulfates **1–3** and a mixture of the sterol sulfates in the proportions found in the sponge inhibited the growth of *B. subtilis* at 100 µg/disc and *S. aureus* at 50 µg/disc but did not inhibit growth of 2 representative marine bacteria. Neither the methyl esters **8–10**, the corresponding hydroxy-acids nor sodium cholesteryl sulfate showed antimicrobial activity at 100 µg/disc, indicating that both the 19-carboxylic acid and 3β-sulfate groups were required for antimicrobial activity. The sterol sulfate mixture inhibited cell division in the fertilized sea urchin egg assay[8] at 5 µg/ml but did not cause cell lysis. The sterol sulfates were toxic to brine shrimp, *Artemia* sp. and goldfish, *Carassius auratus* at 100 µg/ml but not at 10 µg/ml. Even at 150 µg/mg of food pellet, the steroidal sulfates did not act as feeding inhibitors toward goldfish, *C. auratus*, or sergeant major fish, *Abudefduf saxatilis*[9].

In order to determine whether the compounds were being released into seawater, some bioassays were performed using the seawater in which *Toxadocia zumi* had been maintained for 1 h. The water showed antimicrobial properties and caused considerable distress to a keyhole limpet *Megathura crenulata* placed in the water but did not affect the starfish *Pisaster giganteus* or the nudibranch *Anisodoris nobilis*. The seawater also prevented successful development of the polychaete *Salmacina tribranchiata*, as defined by the failure to produce a calcareous tube, but did not affect the larvae of the abalone *Haliotis rufescens* or the ectoproct *Phidolopora pacifica*. The sterol sulfate mixture prevented development of *S. tribranchiata* at 10 µg/ml.

Although it is not feasible to assay compounds against all possible fouling organisms, these results suggest that the sterol sulfates **1–3** might be in part responsible for the lack of fouling organisms on *Toxadocia zumi*. This particular chemical mechanism for protection against fouling is very attractive from a bioenergetic viewpoint, for it involves a 'low cost' synthesis of the biologically active compounds from sterols that are readily available from dietary sources. The only other report of sterol sulfates from sponges is that of the antimicrobial metabolite, halistanol sulfate from *Halichondria* cf. *moorei*[9].

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Effects of bunaphtine on ^{45}Ca movements in rat aortic smooth muscle

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Summary. The effect of bunaphtine (BNA, 5×10^{-5} M) on La^{3+}-resistant ^{45}Ca content and ^{45}Ca efflux was studied on rat aortic smooth muscle. BNA decreased both control and norepinephrine-stimulated La^{3+}-resistant ^{45}Ca content and increased the ^{45}Ca efflux. These effects could explain the inhibition of the contractile responses induced by BNA.

Bunaphtine (BNA) is a new antiarrhythmic drug which has been shown to be a membrane stabilizer[1,2]. In isolated rat thoracic aorta it has been reported that BNA inhibited the contractile responses induced by different agonists and shifted the concentration-response curve of Ca^{++} downwards and to the right[3]. Therefore, these authors suggested

that BNA depressed the contractile response by inhibiting the influx of extracellular Ca^{++} and/or by reducing the release of Ca^{++} from intracellular stores, thus decreasing the availability of Ca^{++} at the contractile apparatus. The present study was undertaken to determine whether BNA could alter the ^{45}Ca movements in the isolated rat thoracic aorta.

Materials and methods. Helically cut strips of rat thoracic aorta were prepared as described by Furchgott and Bhadrakom[4] and suspended under a resting tension of 2 g in 5 ml organ baths containing Tris-Buffer solution (TS) of the following composition (mM): NaCl 136, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.0, glucose 11.0 and tris (hydroxymethyl)aminoethane 6.0. Solutions were adjusted to pH 7.4 with 4.0 N HCl and bubbled with 100% O_2.

^{45}Ca influx was measured as previously described by Godfraind[5]. The aortae were bisected and in each experiment half of the aorta served as control and the other half as experimental preparation. After 2 h of equilibration in TS the experimental half was treated with BNA (5×10^{-5} M) for 5 min. Then both halves were exposed for 5 min to ^{45}Ca-labeled TS (sp.act. 0.5 μCi/ml) already containing norepinephrine (NE) 10^{-6} M. Preparations were then washed for 5 min in Ca^{++}-free TS containing 50 mM $LaCl_3$. After this, strips were removed, blotted on Whatmann No.4 filter paper and weighed. Radioactivity was assayed in a liquid scintillation counter (Intertechnique Model SL-3000) as previously described elsewhere[6].

To determine ^{45}Ca efflux aortic strips were incubated in ^{45}Ca-labeled TS for 2 h and were then placed in successive tubes containing 2 ml of Ca^{++}-free TS every min for the 25-min duration of the washout. In some vials, BNA (5×10^{-5} M) was added to Ca^{++}-free TS. Radioactivity lost into the tubes and present in the tissues at the end of the experiment was measured as described above. The data obtained were plotted as desaturation curves which illustrate the decline of tissue ^{45}Ca with time[7].

Significance of differences in means were tested by Student's t-test.

Results. Figure 1 shows the La^{3+}-resistant ^{45}Ca content after 5 min in ^{45}Ca-TS. The control value for La^{3+}-resistant ^{45}Ca content was 0.050 ± 0.008 mmoles/kg wet weight ($n=8$). BNA, 5×10^{-5} M, reduced the La^{3+}-resistant ^{45}Ca content under control conditions to 0.041 ± 0.007 mmoles/kg wet weight ($n=8$, $p<0.05$). BNA also reduced the La^{3+}-resistant ^{45}Ca content induced by 10^{-6} M NE from 0.218 ± 0.019 to 0.147 ± 0.008 mmoles/kg wet weight ($n=6$, $p<0.001$).

The effects of BNA, 5×10^{-5} M on changes in ^{45}Ca efflux were studied in 5 experiments. Aortic strips were incubated for 2 h in ^{45}Ca labeled TS and then washed out in Ca^{++}-free TS during the first 15 min of the 25-min washout and BNA, 5×10^{-5} M, was added for the final 10 min of the washout. The desaturation curve is shown in figure 2. It indicates that BNA decreased the percentage of Ca^{++} remaining in the aorta by increasing the rate of ^{45}Ca efflux.

Discussion. The concentration of BNA used in this study corresponds to the concentration of the drug required for 50% inhibition of the maximal response to NE, 5-hydroxytryptamine (5HT), KCl and $BaCl_2$[3]. The K^+-induced contractile response is initiated by a depolarization of the cell membrane and subsequent influx of extracellular and/or loosely bound Ca^{++} into the smooth muscle cell[8,9]. This influx of Ca^{++} is also thought to be responsible for the slow component of the NE-induced contractile response[8,10]. On the other hand, the release of Ca^{++} from intracellular stores seems to be the determinant of the fast contractile component of the NE-induced responses[11,12]. 5HT seems to present a similar dependence as NE, whereas $BaCl_2$ is believed to initiate its contractile responses by entering the smooth muscle cell activating the contractile elements and/or releasing Ca from intracellular stores[13]. In the present experiments BNA reduced both control and NE-induced ^{45}Ca influx, which could explain the inhibition of the K^+-induced contractile responses. On the other hand, BNA also increased ^{45}Ca efflux and, as a consequence, it reduced the ability of the smooth muscle cell to store and accumulate Ca^{++}.

All these effects are similar to those reported with local anesthetics in isolated arteries. A variety of drug-induced contractions, including depolarizing concentrations of KCl, of isolated aortic strips can be dose-dependent relaxed by relative high concentrations ($>10^{-5}$ M) of local anesthetics[14]. A decrease in ^{45}Ca influx and an increase in ^{45}Ca efflux have been found in the presence of high concentrations ($>10^{-4}$ M) of procaine[15,16], tetracaine[8,17] and amethocaine[18] in aortic strips. The increase in ^{45}Ca efflux caused by procaine has been attributed[16] to its ability to depolarize the cell membrane. Unfortunately, the effects of BNA on resting membrane potential are unknown in

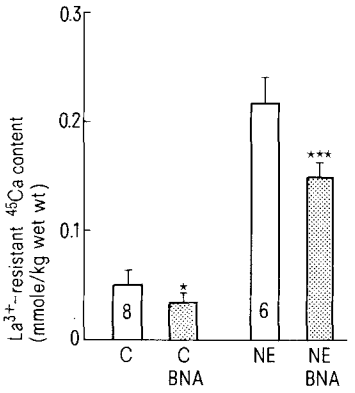

Figure 1. Effect of BNA on La^{3+}-resistant ^{45}Ca content of rat aorta. Preparations incubated for 2 h in TS were transferred for 5 min to ^{45}Ca TS. The effect of BNA, 5×10^{-5} M, on the La^{3+}-resistant ^{45}Ca content was determined in control TS (C) and in TS plus norepinephrine, 10^{-6} M, (NE). Each bar graph is given as mean ± SEM and the number of experiments is shown in the open columns. *$p<0.05$; ***$p<0.001$.

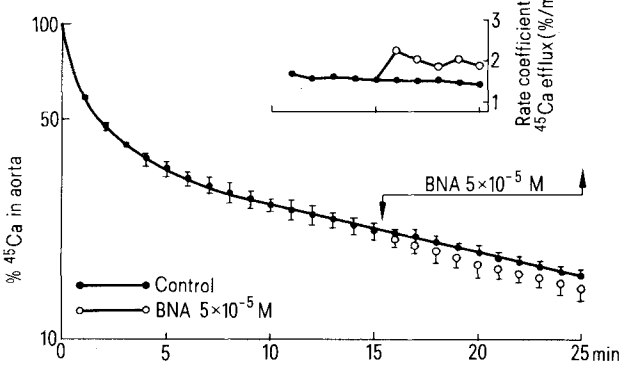

Figure 2. Effect of BNA on ^{45}Ca efflux in rat aorta. Muscles were incubated with ^{45}Ca for 2 h prior to washout in Ca^{++}-free TS. Arrows indicate the interval of exposure to 5×10^{-5} M BNA during the washout. Upper right inset shows the effect of BNA on the rate coefficient of ^{45}Ca efflux from aortic strips. Each data point is given as mean ± SEM ($n=5$).

smooth muscle cells. However, BNA at the concentration used in this study did not depolarize the resting membrane potential in guinea-pig papillary muscles[1].
In conclusion, BNA decreased ^{45}Ca influx and increased ^{45}Ca efflux. Both effects could explain the inhibition of the contractile responses induced by NE, 5-HT, KCl and $BaCl_2$ by BNA in isolated rat aortic strips.

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Failure of naloxone to modify the depth of hypnotic trance

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Summary. Naloxone (10 mg/70 kg, i.v.) was used in normal volunteers to study a possible relationship between endorphins and the depth of hypnotic trance. No effect was found. The drug also failed to modify the subjects' level of alertness.

The endorphins have been implicated, though not always convincingly, in a number of psychic states, including various mental disorders[2,3], acupuncture[4], jogger's high[5-7] and placebo-induced analgesia[8]. One phenomenon of hypnosis, hypnotically induced analgesia, may also involve the endorphins though the conclusion is controversial[9,10]. We decided to investigate the possible participation of endorphins in another aspect of hypnosis, the depth of hypnotic trance. We used the opiate antagonist, naloxone, which blocks the endorphin receptor. If the depth of hypnosis were related to endorphins, then the drug might antagonize the depth of hypnotic trance.
Methods. The subjects were 16 healthy males ranging in age from 19 to 31. They were medical or pre-medical students. They became involved in the experiment because of their interest in hypnosis. All subjects were found to be hypnotizable in preliminary testing which utilized the criteria of Spiegel[11] and Spiegel and Fleiss[12]. Written consent forms and health questionnaires were administered. There were no indications of illness. The subjects were questioned about their use of psychoactive drugs. Most admitted an occasional use of alcohol and marijuana, but denied using other agents. They agreed to refrain from alcohol and marijuana for at least 72 h prior to each experimental session. The subjects were informed that after screening, they would be hypnotized by a psychiatrist skilled in hypnosis on 3 occasions following the i.v. administration of either naloxone or placebo during 2 of the trials. The remaining trial (no injection) would serve as a baseline.
The subjects were randomly divided into different groups, based on the possible sequences of naloxone, placebo and baseline. The placebo was the drug vehicle, as supplied by the manufacturer. During the baseline (no injection) sessions, as in sessions involving actual injections, a small bandage was placed on the subjects' forearm to prevent the hypnotist from recognizing whether or not an injection had been given. Only the person giving the injections was aware of the sequences of the treatments. The placebo was given in a dose of 1 ml/70 kg, i.v. The dose of naloxone was 10 mg/ml/70 kg, i.v. Although this dose is well above the standard clinical dose used to treat narcotic overdose, no side effects have been reported in the literature at this level[13]. The high dose was chosen because of evidence that some effects attributed to endorphins appear to be blocked only by large doses of naloxone and because of the efficacy of such doses in certain behavioral conditions in humans[2]. After treatment, a 15-min waiting period was allowed, to permit drug equilibration. After the waiting period, the subjects were hypnotized and the depth of trance was assessed according to the Hypnotic Induction Profile of Spiegel[11,12]. This test provides a score which measures the depth of hypnotic trance. The scores range from 0 (subjects who are refractory to hypnosis), to 10 (subjects who are hypnotizable and who experience a very deep trance). Spiegel and Fleiss[12] found that the mean score for a sample of 1674 normal subjects was 6.7 ± 2.9. Following the session with the hypnotist, the subjects were asked to rate their level of alertness according to the Stanford Sleepiness Scale[14]. This questionnaire provides a rating of sleepiness with scores ranging from 1 (fully alert) to 7 (almost asleep). The subjects were also asked to describe any subjective effects and whether they could identify when they received drug or placebo.

Measures of the depth of hypnotic trance and of sleepiness after the induction of hypnosis in subjects receiving no prior treatment (baseline), or pretreated with placebo (1 ml/70 kg, i.v.) or naloxone (10 mg/ml/70 kg, i.v.)

	Baseline	Placebo	Naloxone
Hypnotic induction Profile score	7.1 ± 0.4	6.2 ± 0.6	6.0 ± 0.8
Stanford sleepiness Scale score	2.5 ± 0.2	2.6 ± 0.6	2.6 ± 0.3

The values shown are means, $\pm$SE. N = 16.