dian hypothalamic nuclei (nucleus preopticus pars magnocellularis and pars parvocellularis, nucleus lateralis tuberis[17]) have been observed, whereas no labelling occurred in neurons of the nucleus periventricularis recessus lateralis. In addition, a neurosecretory function of cells bordering the lateral recessus of the inferior lobes of *Lepomis cyanellus* and *Carassius auratus* has been proposed from electron microscopic evidence[10].

The fact that the ventricular channel-system has pores seems to be unique among adult vertebrates. Openings in the neural tube of vertebrates are only known in transitional embryonic stages as neuroporus. This could affect a structural component of the blood-liquor barrier. The latter includes in general in the choroid plexus systems an endothelial (mesodermal) as well as an epithelial (neuroectodermal) component. Possible functions of a presumptive blood-liquor barrier (in the venous network) would therefore be either limited to the venous endothelium (as in the mammalian leptomeninx) or supplied at the ventricular ependyma (by a liquor-brain barrier).

Many advanced teleosts among perciform and tetraodontiform fish show a great ontogenetic plasticity of the following phenomena[19-22]: growth and therefore size, social function and sex change. These features show a remarkable contrast to land vertebrates[23]. Moreover, these developmental changes have been clearly demonstrated in *Amphiprion* species to depend on the dynamics of the social system in relation to the environment[20,22]. Optical clues seem to play a predominant role in these biological changes.

However, a speculative idea could be admitted at this point: advanced teleost fish developed the complex vegetative regulatory systems in a new way. In addition to the hypophysis some advanced teleost fish possess a second area that can act as a bridge between neuronal and humoral processes.

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Crown ethers which influence cardiac and respiratory muscle contractility[1]

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Summary. Guinea-pig tracheal smooth muscle and heart muscle demonstrated a variety of in vitro positive and negative inotropic responses to concentrations of crown ethers in the nmole/l to μmole/l range. It is suggested that these ionophoretic compounds have potential as therapeutic agents.

The chemistry of crown ethers has developed rapidly in the years since Pedersen first described their synthesis in 1967[4]. These macrocyclic polyethers readily bind a wide range of cations including such physiologically important ions as Ca^{++}, Na^+, and K^+. In that cations of appropriate size fit into the heteroatom binding site in the crown ether ring, alterations in either ring size or side chain structure can alter the intensity of ionic binding and result in a diversity of physicochemical properties[5-11]. Frensdorff[12] described the ionophoretic properties of crown ethers, and demonstrated the ability of these compounds to selectively transport ions from aqueous solutions across hydrophobic membranes by a process of carrier translocations. Behr and his coworkers[13] recently reported that solid-state models of biological transmembrane channel complexes consist of groups of functionalized macrocyclic polyethers.

With the exceptions of the work of Gunther et al[14] and Achenbach et al.[15] who studied cryptands as possible Na^+ and K^+ ionophores in cardiac purkinje fibers, little informations has been published concerning the physiological effects of crown ethers on muscle or upon the ionic transport mechanisms responsible for the development of muscle tension. The present studies examined a selected group of crown ethers in an attempt to discern possible structure function relationship in 2 in vitro muscle systems. The guinea-pig tracheal smooth muscle[16] and perfused heart muscle demonstrated a wide range of positive and negative contractile responses to crown ethers.

Materials and methods. Male Hartley guinea-pigs weighing 350–450 g were killed by a sharp blow to the head. Following midsternal thoracotomy, both the trachea and the heart were rapidly excised and immersed in cold oxygenated physiological medium the composition of which was as follows (mmole/l): Na^+ 126.9; K^+ 5.8; Ca^{++} 2.5; Mg^{++} 1.2; Cl^- 127.7; HCO_3^- 8.9; $H_2PO_4^-$ 1.1; and glucose 11.0.

Tracheal ring segments. Extraneous tissue was removed from

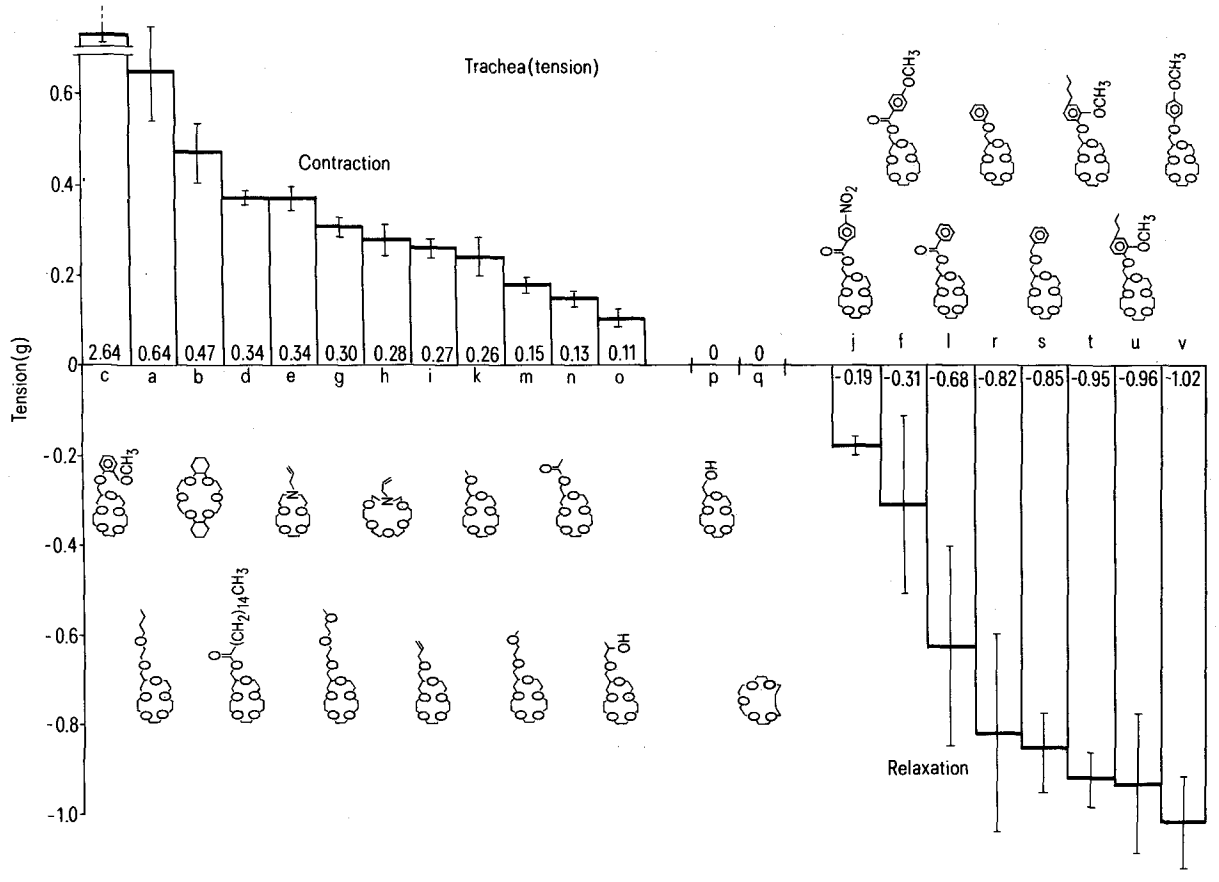

Figure 1. Maximal increase or decrease in tension (g) of incubated guinea-pig tracheal rings exposed to various crown ether analogs. Control line represents a 2 g equilibration tension. The data are expressed as the mean ± SEM of 7 to 21 studies. The crown ether codes are identified in the table.

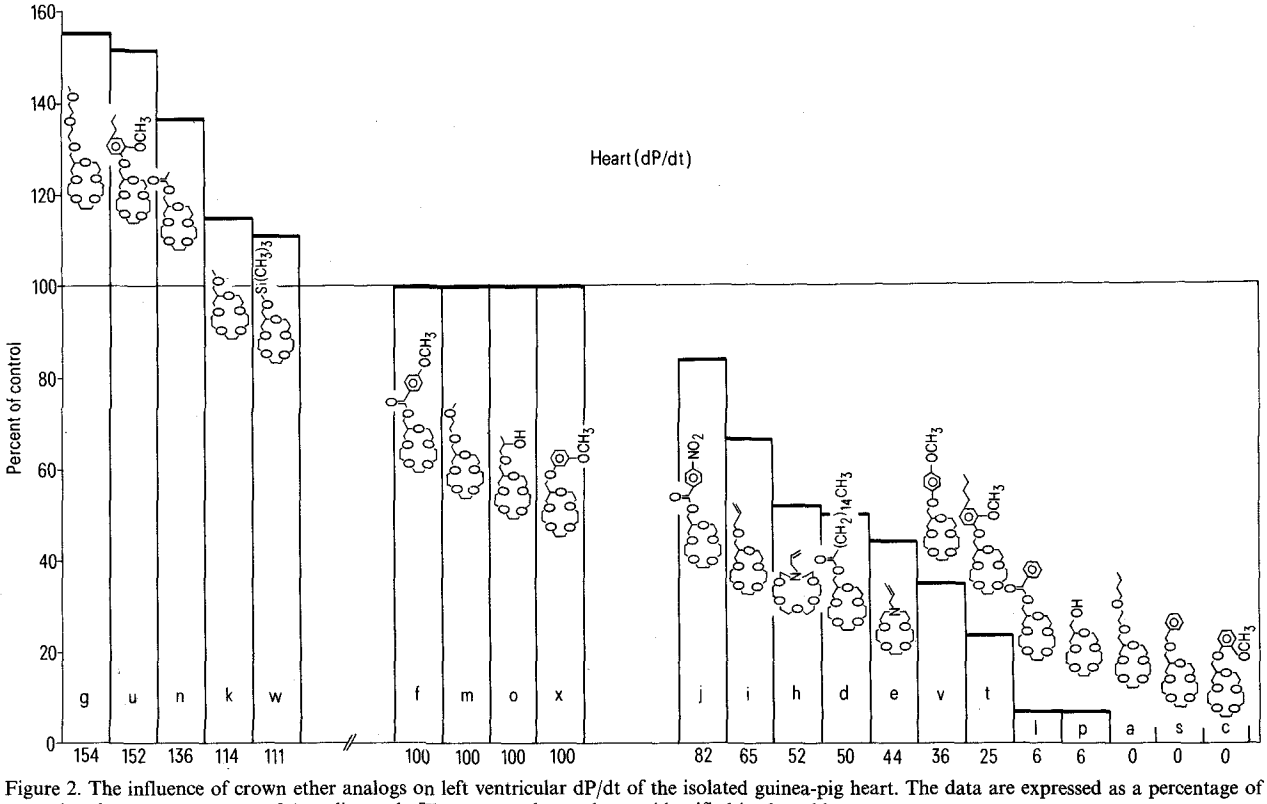

Figure 2. The influence of crown ether analogs on left ventricular dP/dt of the isolated guinea-pig heart. The data are expressed as a percentage of control and represent a mean of 4 studies each. The crown ether codes are identified in the table.

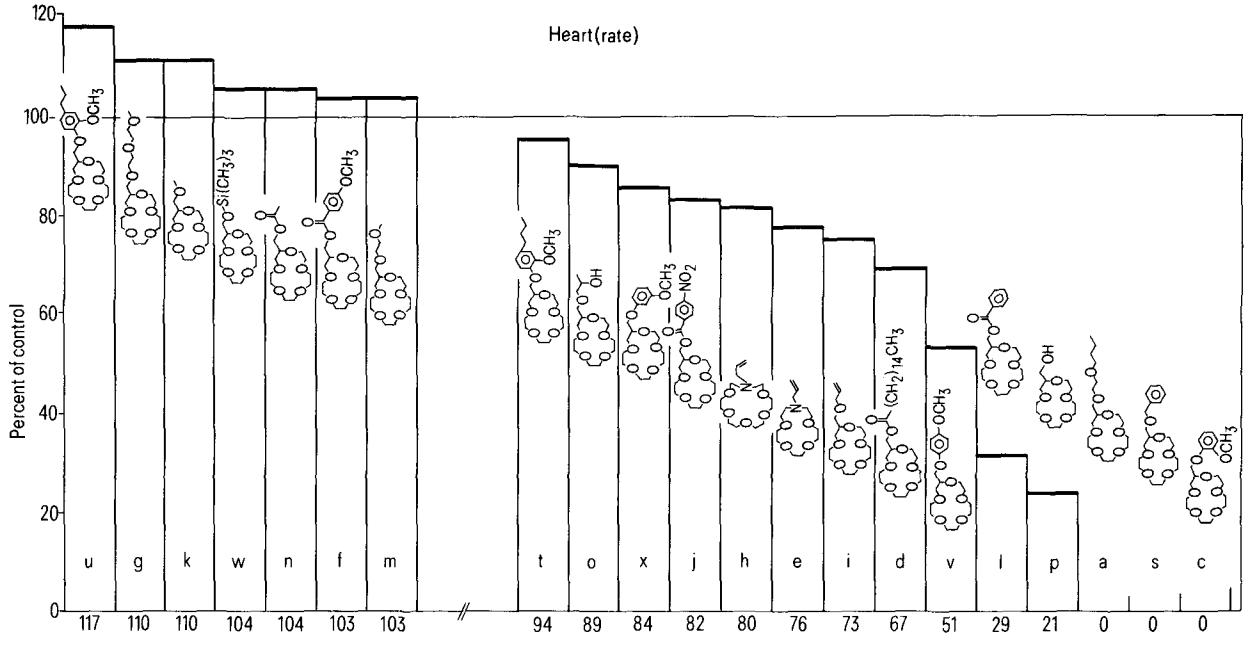

Figure 3. The influence of crown ether analogs on left ventricular contractile rate of the isolated guinea-pig heart. The data are expressed as described in the legend of figure 2.

each tracheae and they were sectioned into 4 mm rings which were suspended in 20 ml incubation chambers according to the method of Hooker et al[17]. During incubation the physiological fluid was constantly bubbled with a mixture of 95% O_2 and 5% CO_2 so as to maintain the pH at 7.4. The temperature was held constant at 37 °C. After 1 h of equilibration under a preload tension of 2 g (which was determined to be the 'optimal' tension necessary to assure maximum response and stability of the tracheal preparations), log concentration-response curves were determined for each crown ether. Incremental amounts of the crown ethers were added to the baths on a cumulative basis, allowing sufficient time to lapse between successive additions for equilibration of tension (usually less than 10 min).

Isolated perfused hearts. Immediately after excision myocardial perfusion was initiated through the severed ascending aortae of each isolated heart via small perfusion tubes secured in place with surgical ligatures. The perfusion fluid was presented to the hearts at a constant pressure of 60 cm H_2O and at a constant flow rate of 15 ml/min, a rate slightly in excess of the needs of the heart with superfluous fluid being shunted to a reservoir. The actual flow rate through the coronary arteries varied according to the demands of the heart as determined by a number of factors, including heart rate, myocardial contractile force and coronary resistance. The measured flow rate, however, usually fell in the range of 7 to 12 ml/min and hearts whose perfusions rate exceeded 15 ml/min were eliminated from the study. The hearts were allowed to beat spontaneously without electrical pacing. A 5F Swan-Ganz diagnostic balloon tipped catheter was inserted into left ventricular chamber via the apical dimple and filled with 0.5 ml of water. The pressure

Crown ether analogs used in this study

Code	Name	Effective concentration range (M)	Ref.
a)	2,5-dioxanonyl-15-crown-5	$7 \times 10^{-7} - 2 \times 10^{-4}$	20
b)	Dicyclohexano-18-crown-6	$2 \times 10^{-6} - 5 \times 10^{-5}$	21
c)	*o*-methoxyphenoxymethyl-15-crown-5	$8 \times 10^{-7} - 9 \times 10^{-5}$	20
d)	Hexadecanoylmethyl-15-crown-5	$5 \times 10^{-7} - 5 \times 10^{-4}$	22
e)	N-allylmonoaza-15-crown-5	$1 \times 10^{-6} - 6 \times 10^{-4}$	22
f)	*p*-methoxybenzoylmethyl-15-crown-5	$1 \times 10^{-9} - 1 \times 10^{-4}$	22
g)	2,3,8-trioxanonyl-15-crown-5	$2 \times 10^{-6} - 6 \times 10^{-4}$	22
h)	N-allylmonoaza-18-crown-6	$1 \times 10^{-6} - 6 \times 10^{-4}$	23
i)	Allyloxymethyl-15-crown-5	$7 \times 10^{-7} - 5 \times 10^{-4}$	24
j)	*p*-nitrobenzoylmethyl-15-crown-5	$8 \times 10^{-7} - 4 \times 10^{-4}$	22
k)	Methoxymethyl-15-crown-5	$1 \times 10^{-6} - 2 \times 10^{-3}$	20
l)	Benzoylmethyl-15-crown-5	$2 \times 10^{-6} - 8 \times 10^{-4}$	20
m)	2,5-dioxyhexyl-15-crown-5	$1 \times 10^{-9} - 1 \times 10^{-4}$	22
n)	Acetoxymethyl-15-crown-5	$6 \times 10^{-7} - 1 \times 10^{-3}$	22
o)	2-hydroxypropyloxymethyl-15-crown-5	$1 \times 10^{-9} - 1 \times 10^{-4}$	22
p)	Hydroxymethyl-15-crown-5	$8 \times 10^{-7} - 3 \times 10^{-4}$	25
q)	15-crown-5	$1 \times 10^{-9} - 1 \times 10^{-4}$	24
r)	Phenoxymethyl-15-crown-5	$1 \times 10^{-5} - 1 \times 10^{-4}$	22
s)	Benzyloxymethyl-15-crown-5	$1 \times 10^{-6} - 8 \times 10^{-5}$	22
t)	4-allyl-2-methoxyphenoxymethyl-15-crown-5	$6 \times 10^{-7} - 3 \times 10^{-4}$	22
u)	(2-methoxy-4-propyl) phenoxymethyl-15-crown-5	$1 \times 10^{-6} - 1 \times 10^{-4}$	22
v)	4-methoxyphenoxymethyl-15-crown-5	$2 \times 10^{-6} - 7 \times 10^{-5}$	20
w)	Trimethylsiloxymethyl-15-crown-5	$1 \times 10^{-6} - 5 \times 10^{-5}$	22
x)	*m*-methoxyphenoxymethyl-15-crown-5	$1 \times 10^{-9} - 1 \times 10^{-4}$	22

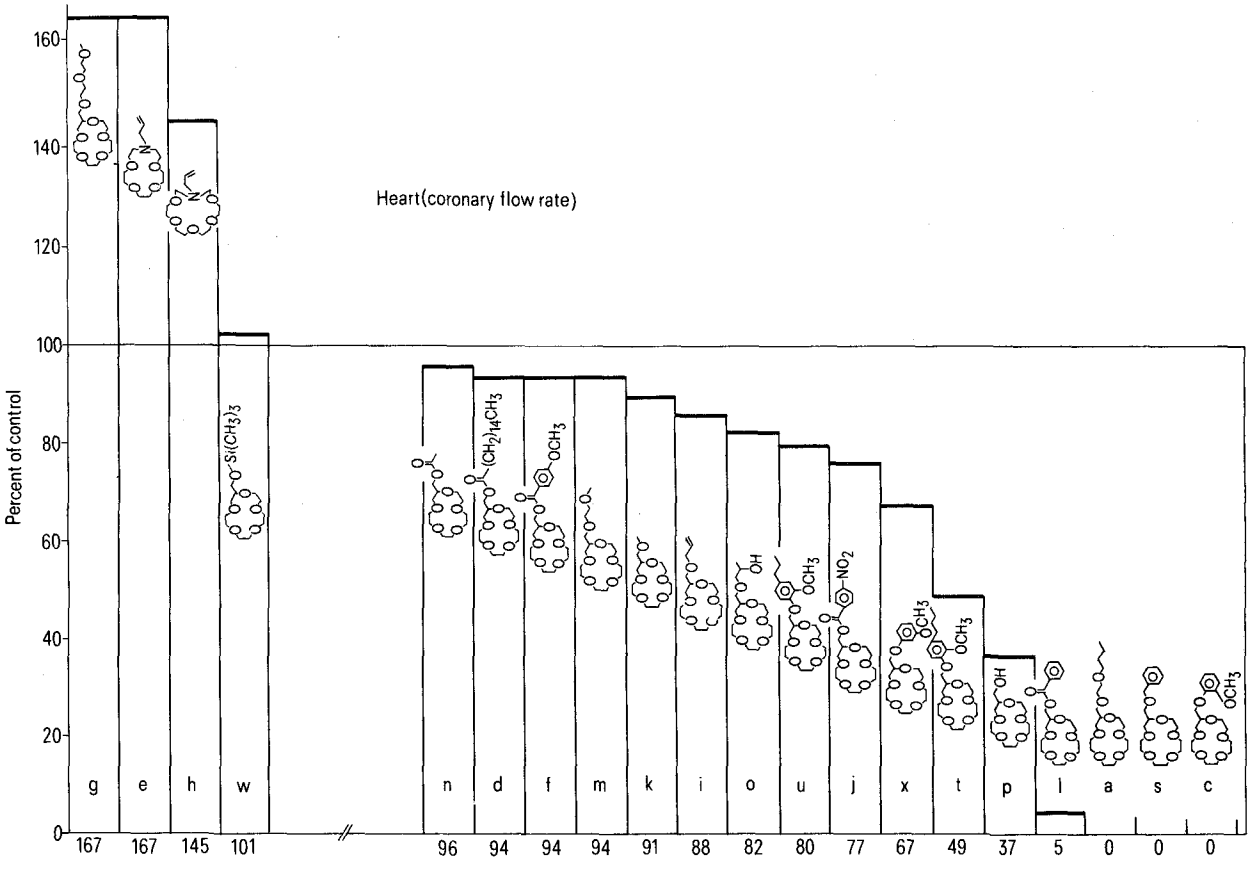

Figure 4. The influence of crown ether analogs on the coronary perfusion rate of the isolated guinea-pig heart. The data are expressed as described in the legend of figure 2.

pulse of each contraction of the heart was transmitted to the balloon and from there to a low volume displacement pressure transducer. Signals from the pressure transducer were recorded on a physiological recorder as the first derivative of the pressure pulse (dP/dt) inorder to obtain an index of contractile strength in a rapidly contracting muscle. In each experiment, after 30 min of equilibration, a syringe pump was used to slowly infuse a selected crown ether into the main perfusion line. In that the perfusion pump rate remained constant at 15 ml/min it was possible to adjust the crown ether infusion rate so as to achieve a desired perfusion fluid concentration with accuracy. Each perfused heart was exposed to increasing increments of crown ethers with sufficient time lag for equilibration of contraction between successive increments (usually less than 10 min).

Concentration-response. In each experiment the tissues were exposed to increasing concentrations of 1 particular crown ether until no further change in response could be note (see table). The effects of the crown ether analogs used in this study appeared to be readily reversible. All contractile parameters returned toward control conditions when the crown ether infusion was stopped. The data recorded in figures 1–4 denote maximal responses of the tissue to each crown ether as compared to appropriate control values. The maximal increase or decrease of tracheal tension from the 2 g equilibrium value is recorded in figure 1. The data derived from the perfused heart experiments (figures 2–4) are expressed as a percentage of control and were determined by dividing the specific maximal response values by comparable control values.

The crown ether analogs used in this study are listed in the table. Except for analogs b and h which are 18-crown-6 compounds, all the crown ethers utilized in this study are derivatives of 15-crown-5. The data of figure 1 represent mean values ± SE of the mean and are a composite of 7 to 21 experiments. The data of figure 2–4 are expressed as a percentage of control and represent a mean of 4 experiments each.

Results and discussion. The responses of the incubated guinea-pig tracheal cylinders to the various crown ethers are shown in figure 1. Crown ethers such as 2,5-dioxanonyl-15-crown-5 (a), with polyether side chains, enhanced tracheal contractility. Crowns such as 4-methoxyphenoxymethyl-15-crown-5 (v), on the other hand, with aromatic side chains, diminished tracheal contractility. In that polyether side chains diminish crown ether lipophilicity and aromatic side chains enhance crown ether lipophilicity, these results would suggest a potential inverse-relationship between crown ether lipophilicity and tracheal contractile response.

The responses of the perfused hearts to the crown ether analogs are shown in figures 2–4. Expressed as a percentage of control, the contractility results (fig. 2) indicate that 2, 3, 8-trioxanonyl-15-crown-5 (g) was one of a group of compounds which enhanced myocardial dP/dt while 2, 5-dioxanonyl-15-crown-5 (a), benzyloxymethyl-15-crown-5 (s), and o-methoxyphenoxymethyl-15-crown-5 (c) were members of a group which suppressed contractile activity. Several crown ethers did not influence myocardial dP/dt at all. The relative order of potency of the crown ethers in altering myocardial contractile rate (fig. 3) is closely related to the order noted for dP/dt (fig. 2). The order of potency of the crown ethers in altering coronary flow (fig. 4), however, is only marginally related to the order noted for dP/dt (fig. 2), a finding probably indicative of smooth muscle involvement. No straight forward structure-

function relationships could be determined for crown ethers in the perfused hearts. The responses to crown ether (2-methoxy-4-propyl) phenoxymethyl-15-crown-5 (u) are of particular interest. This compound increased myocardial dP/dt while relaxing tracheal smooth muscle at μmole/l concentrations.

Clearly, further investigation is necessary to completely characterize the effects of the various crown ethers on muscle contractility. Although the biological mechanisms of action of these compounds are not clearly understood, we suggest that since they are will known as cation complexing agents, their effects on smooth muscle and cardiac muscle may involve effects on transmembrane Na^+ and K^+ fluxes and/or on intracellular shifts in the concentration of Ca^{++}, as have been noted with other inotropic agents[18,19]. Benninger et al.[20] clearly described the close relationship between transmembrane cation fluxes, particularly between Ca^{++} and Na^+.

It would seem appropriate that further studies should focus on the influence of crown ethers on in vivo cardiopulmonary systems and on possible in vivo toxicity. Our findings suggest that the potential of crown ethers as therapeutic agents should be investigated.

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3 Also members of the Department of Endocrinology.

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Morphine eye-drops reduce homatropine induced mydriasis in man[1]

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Summary. In 7 healthy volunteers 4% morphine eye-drops, when administered to one eye, caused a miosis limited to that eye. In 7 other healthy volunteers morphine was administered into one eye after bilateral instillation of 0.5% homatropine opthalmic drops; the eye treated with morphine and homatropine showed a mydriasis less intense than the other eye treated only with homatropine. It is suggested that topical morphine locally affects sympathetic function by inhibiting noradrenaline release into the iris neuromuscular junction.

It is generally accepted that the miotic action of morphine is mediated entirely by means of activation in the central nervous system of the parasympathetic tone of the iris[2-4]. However, the capacity of topical morphine in humans to induce moisis suggests a direct action of the drug on the iris neuromuscular junction[5,6]. Furthermore, topical morphine-induced moisis is reversed by topical naloxone, thus suggesting the existence of opiate receptors in the human iris[6]. On the other hand, it still remains to be defined whether morphine acts directly on the iris muscle or through pupillary branches of the autonomic nervous system. In fact, morphine has a very specific effect on certain peripheral autonomic neuroeffector junctions which appear to be modulated by opiates[7-9]. In particular, animal data show that morphine inhibits noradrenaline (NA) release[10-12]. In the current study we have assumed as a working hypothesis that iris opiate receptors modulating NA release are located presynaptically on the adrenergic synapses. In order to verify this assumption we have evaluated the effect of topical morphine on both the pupil size and the mydriasis induced by the cholinoceptor blocker homatropine.

Materials and methods. 14 healthy volunteers (10 ♂ and 4 ♀) aged between 31 and 62 years (mean ± SEM = 35.46 ± 2.73) participated in this study. The purpose and procedure of the investigation were thoroughly explained to all subjects and informed consent was obtained. All volunteers were drug-free for a period of at least 2 weeks prior to the study. All tests started at 09.00 h.

7 subjects (4 ♂ and 3 ♀) ranging in age between 31 and 59 years (mean ± SEM = 46.3 ± 3.5), received 2 drops (0.1 ml) of a 4% aqueous solution of morphine hydrochloride or 2 drops of a saline solution into right and left conjuctival sacs respectively. One drop (0.05 ml) of a 0.5% homatropine solution was in-