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.

1 We thank Drs G.W. Gokel, D.M. Dishong, and C.J. Diamond of the Department of Chemistry, University of Maryland, College Park, Maryland, for constructive criticisms, helpful advice, and careful review of the manuscript, and for providing many of the compounds used in this study. We also thank the W.R. Grace Co., which supported the development of most of the compounds.

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22 These compounds were generously provided by G.W. Gokel, Department of Chemistry, University of Maryland, and by W.R. Grace & Co., which supported his research.

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0014-4754/84/07/727-05$1.50 + 0.20/0

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-

Influence of morphine eye drops on pupil size and on homatropine-induced mydriasis

Basal values (n = 7)	mm (mean ± SEM)		Time after instillation (min) 30	60	90	120	150	180
Right eye (morphine)	4.3 ± 0.5	p	NS	< 0.01	< 0.001	< 0.001	< 0.001	< 0.05
Left eye (saline)	4.3 ± 0.5		NS	NS	NS	NS	NS	NS
Right eye (homatropine plus morphine)	4.2 ± 0.1	p <	0.001	0.001	0.001	0.001	0.001	0.001
Left eye (homatropine plus saline)	4.2 ± 0.1		0.001	0.001	0.001	0.001	0.001	0.001

p versus basal values.

stilled into both eyes of 7 subjects (6 ♂ and 1 ♀) ranging in age from 31 and 62 years. The solution of homatropine pH 7 in physiological saline was prepared with a buffer phosphate.

5 min after homatropine instillation, 2 drops of 4% aqueous solutions of morphine or saline were instilled into the right and left conjunctival sacs respectively. During each test, after drug administration, the ipsilateral nasolacrimal duct was occluded for 2 min using digital pressure. Subjects were then requested to blink 2 or 3 times slowly. Pupillary diameters of both eyes were measured under standard light conditions using a photographic technique. Pupils were photographed with 2 Nikkormat FT2 single lens reflex cameras using 1 camera for each eye. A macrophoto of the pupils in the ratio of 1:1 was obtained. It was possible to measure the transverse diameter of the pupil directly on the negative[13].

Pupil diameters of both eyes were measured prior to drug instillation and every 30 min thereafter for a period of 3 h. Basal pupil diameter was expressed in mm, and changes after treatment as a percentage of the baseline value.

Statistical analysis was performed by means of Student's t-test for paired data. All subjects completed the experimental session.

Results. Morphine test. Morphine eye-drops induced a significant miosis both when compared with pre-drug values and with the contralateral eye treated with saline eye-drops (fig., table).

Homatropine-morphine test. The time-course of changes in pupil diameter following instillation of eye-drops is showed in the figure. In both eyes, homatropine induced a mydriasis statistically significant for the entire period of observation (table). However, the mydriatic effect in the eye treated with both homatropine and morphine was significantly smaller than that obtained in the eye treated only with homatropine (fig.). The difference between the 2 eyes was significant from 60 to 180 min after homatropine.

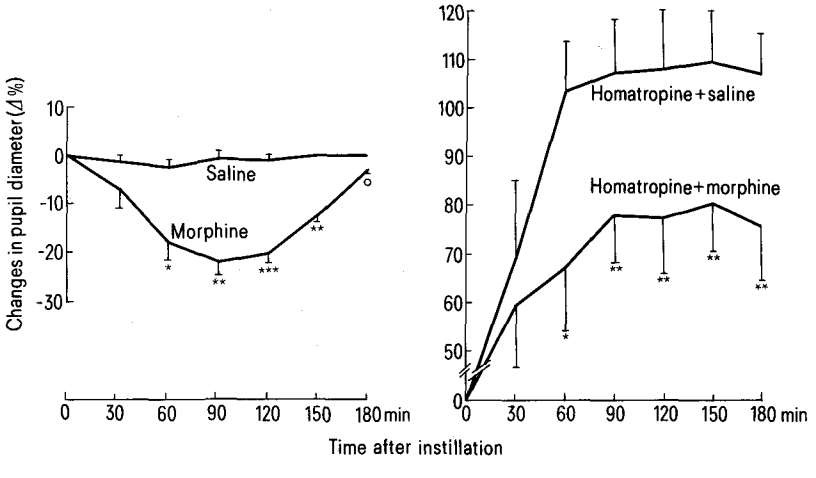
Discussion. The results of this study show that morphine eye-drops induce moisis. This is apparently a local effect, as the miosis is limited only to the morphine-treated eye. Furthermore, morphine eye-drops are capable of reducing the homatropine-induced mydriasis, also possibly due to the local action of morphine.

Although it cannot be excluded, it seems unlikely that morphine acts directly on the sphincter muscle by inducing a constriction despite the cholinoceptor blockade. Since morphine and endogenous opioids mainly exhibit an inhibitory action, the system affected by local morphine is likely to be the sympathetic neuromuscular junction. In fact, it has been postulated that the miotic effect of morphine when systemically administered results from an inhibition on the catecholaminergic fibers which normally control the activity of the preganglionic parasympathetic neuron which in turn originates in the central nervous system and innervates the sphincter muscle of the eye[14]. Our findings therefore, support the fact that conjunctival morphine provokes moisis by stimulation of opiate receptors located on the iris noradrenergic ending. In fact, homatropine induces a block of the iris cholinoceptor and the consequent mydriasis is exclusively due to an unopposed sympathetic tone. Conjunctival morphine, therefore, may cause an inhibitory action on sympathetic transmission, thus reducing homatropine-induced mydriasis.

The miotic effect of local morphine reaches a significant level ($p < 0.01$) 60 min after eye-drop administration. The latency is possibly due to the slow passage through both the conjuctival membrane and the anterior chamber before acting on the iris neuromuscular junction. This could be verified by estimating morphine levels in the eye by humor aqueous aspiration, but this invasive technique cannot be carried out in healthy volunteers.

The iris neuromuscular junction, therefore, may be an example of opiate-modulated adrenergic neurotransmission in man. The human venous wall represents a similar example as it appears to contain morphine-sensitive adrenergic junctions[15].

In view of these findings, it seems possible that the mydriasis following conjunctival naloxone instillation observed in mor-



Left 2 eye-drops of a 4% morphine solution induced a significant miosis when compared with the saline-treated eye. Mean ± SEM; n = 7. Right Pupillary changes after a single drop of a 0.5% homatropine solution in both eyes, followed, after 5 min, by 2 drops of a 4% morphine solution in one eye and 2 drops of a saline solution in the other. Homatropine induced mydriasis was significant for the entire period of observation. The mydriasis in the eye treated with homatropine plus morphine was significantly smaller than that observed in the eye treated only with homatropine. Mean ± SEM; n = 7. Significance between the 2 pupils: *$p < 0.01$; **$p < 0.001$; ***$p < 0.005$; °$p < 0.05$ (Student's t-test).

phine addicts[16,17] could be mediated by the release of noradrenaline accumulated during morphine abuse. The fact that conjunctival naloxone had no effect on the pupil size of healthy volunteers not treated with morphine[16] or treated with a single clinical dose of morphine[6] does not exclude the possibility that the iris opiate receptors take part in the physiological control of the pupillary size.

A miosis, reversible by naloxone eye-drops, has in fact been observed in man after strenuous physical exercise[18]. This fact confirms the opinion that the activity of the natural opioid system, 'silent' in its basic condition, is operant in stressful conditions capable of affecting homeostatic balance.

1 This work was partly supported by the CNR study group 'Pain Control'. The authors wish to thank Dr Tendi of the Pharmacy of St. Maria Nuova Hospital, Florence, for the preparation of the eye-drops.

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0014-4754/84/070731-03$1.50 + 0.20/0

Arginine has a morphine-like action in insects

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Summary. Injections of arginine and morphine increased the voltage of electric shocks necessary to induce a defensive reaction in the Praying Mantis. The effect of both was inhibited by naloxone.

Recently it has been shown that morphine has an antinoxious action in 2 arthropods: the mantis shrimp[2] and the honey bee[3]. Morphine increases the threshold for behavioral responses to electric shocks, i.e. the violent flexure of the body of the shrimp and the extrusion of the sting in the bee; and naloxone, if it is injected together with morphine, inhibits this action. It is also known that morphine and endorphines affect memory formation in vertebrates[4], but no report is known for arthropods. In the praying mantis *(Stagmatoptera biocellata)* it has been shown that the levels of arginine in the cerebral ganglia increased in those insects which learned not to attack a moving star or not to display a defensive reaction (the deimatic reaction[5]). This increase in the level of arginine was not due to the training activity in itself but appeared only in those animals which later retained the learned task[6]. Injections of arginine before training induced in this insect consolidation of long term memory in training situations in which otherwise formation of long term memory never have occurred[7]. Therefore, in this work we compare the antinoxious effect of arginine to that of morphine in the praying mantis, in order to prepare for future investigation of the relation between morphine and memory.

Praying mantises display a defensive response, i.e. a deimatic reaction[5] when they are touched, presented to a bird, or submitted to electric shocks on their legs, abdomen, thorax or head. Electric shocks of increasing voltage were given until the mantises displayed a full deimatic reaction[8]. For this purpose, 96 female mantises (12 for each dose tested), were fixed on a mantis holder, and a teflon cannula was implanted chronically into the thoracic cavity in order to make the injections[7]. The injected volume was always 50 µl. Two stainless steel electrodes were implanted chronically into the epicranial sklerite to the extent just necessary to perforate the cuticle. Through them, square bimodal electric pulses (1 msec duration and 100 Hz) of increasing voltage were applied until a full deimatic reaction was observed[8]. 1 min after measuring this first threshold, the insects were injected with the drug to be tested, and the voltage threshold was again measured at 1, 2 and 4 h after the injection of the drug. This new voltage could be either 0, 25, 75 or 100% higher than the voltage threshold measured before the injection. If no response was elicited with a 100% voltage in-

Percentage of stimulus threshold increase necessary to produce a full deimatic reaction after various types of injections

Amount injected (mg/g of insect)		Stimulus threshold (%) after injection Median (range) n = 12 after 1 h	2 h	4 h
Arginine	+Naloxone			
0	0	0 (0– 25)[a]	0 (0-25)[a]	0 (0–25)[a]
3.5	0	25 (25– 75)[b]	25 (0– 50)[b]	0 (0–25)[a]
5.0	0	100 (0–100)[c]	50 (25–100)[c]	25 (0–50)[b]
6.5	0	100 (100–100)[c]	87 (75–100)[d]	50 (0–75)[b]
5.0	0.032	37 (25– 75)[b]	25 (0– 50)[a,b]	0 (0–25)[a]
Morphine	+Naloxone			
0.35	0	100 (0–100)[c]	50 (0–100)[c]	0 (0–75)[b]
0.35	0.032	25 (0–100)[b]	0 (0–100)[a,b]	12 (0–25)[a]
0	0.032	12 (0– 75)[a,b]	25 (0– 50)[a,b]	25 (0–25)[a]
Analysis of variance H		49.80	37.12	25.56
(Kruskal-Wallis) p		< 0.001	< 0.001	< 0.001

[a,b,c] and [d] indicate statistically different medians for each column given by the Mann-Whitney U test, $\alpha = 0.05$.