decreased r-RNA synthesis, in contrast to dramatic increases previously described in regenerating neurons[5]. When the nucleolar response of axotomized neurons is insufficient, delayed axonal regrowth and cell death are known to occur[6]. This finding may explain the present observation of increased neuronal degeneration and that of decreased axon outgrowth[7] after chronic morphine administration. Morphine-induced alterations of RER ultrastructure observed in unoperated and 3-day regenerating neurons resembled the in-vitro disattachment of membrane-bound ribosomes which occurs after puromycin-high salt treatment[8]. Similar 'narrowed' ER cisternae have been reported in dorsal root ganglion neurons after treatment with aluminium phosphate[9] and in chromatolytic spinal motor neurons[10]. However, these membrane complexes appeared to originate from smooth ER and, unlike the morphine-altered RER, cisternae were closely opposed and lacked an intercisternal matrix. Since morphine administration significantly inhibited protein synthesis in normal[2] and axotomized[4] neurons, flattened cisternae may be interpreted as the morphological expression of decreased synthetic activity following drug-induced ribosomal disattachment.

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The inhibitory effect of paraquat on histamine and isoproterenol induced changes of cyclic nucleotides in rat lung slices[1]

S.N. Giri and M.A. Hollinger

Departments of Physiological Sciences and Pharmacology, University of California, Davis (California 95616, USA), 20 November 1978

Summary. The incubation of rat lung slices with paraquat ion (10^{-4} M) had no effect on cAMP and cGMP levels of the rat lung slices. The preincubation with the same concentration of paraquat inhibited the cAMP elevating effect of histamine (10^{-5} M) and isoproterenol (10^{-5} M) and reduced the cGMP level to approximately 50% of the level obtained without preincubation with paraquat.

It is known that cyclic nucleotides content, particularly, the cGMP of mammalian lung is high relative to that of most other tissues[2,3]. The cyclic nucleotides are thought to be involved in various physiological and pathological processes of tissues. Evidence from several sources indicate that cyclic nucleotides and agents that influence their concentration in the lung can play an important role in the regulation of metabolism and perhaps specific cell function[4]. In view of a regulatory role that cyclic nucleotides can play on various aspects of lung cell function, we have studied the effects of the herbicide paraquat, a lung toxicant[5], on cyclic nucleotide levels of the rat lung slices and its interaction with the drugs histamine[6] and isoproterenol[7] That are known to elevate the cyclic nucleotide levels of the lung slices.

Methods. Certified pathogen-free, male Sprague-Dawley rats weighing 280–340 g were used.The rats were sacrificed by ether and exanguination. The lungs were perfused in situ with cold isotonic saline at 4–5 °C through the right side of the heart[8]. The individual lung lobes were excised and transferred into a beaker containing ice-cold Krebs Ringer Bicarbonate Buffer (KRBB) which was previously gassed with 5% CO_2+95% O_2 (v/v) for 15 min. The lobes were washed with KRBB to remove extraneous tissue. The slices from each lung lobe were obtained in a cold room at 4–6 °C using a Stadie-Riggs microtome. The slices from the lungs of 6–8 rats after washing with KRBB were pooled together in a beaker containing KRBB previously gassed with 5% CO_2+95% O_2 (v/v). Approximately 450–500 mg lung slices were transferred into each reaction vessel containing 2.9 ml fresh KRBB and 10 mM theophylline. The reaction vessels were preincubated for 15 min at 37 °C in an atmosphere of 5% CO_2+95% O_2 (v/v). At the end of preincubation 0.1 ml of paraquat dichloride solution dissolved in KRBB, was added to the reaction mixture to give a final concentration of paraquat ion 10^{-4}M. The control samples received 0.1 ml of KRBB. The slices were incubated for 2, 4, 6 and 12 min after addition of paraquat or KRBB under an atmosphere of 5% CO_2+95% O_2 (v/v).

In some experiments, slices were first preincubated in the presence of paraquat ion (10^{-4} M) and incubation was started after the addition of histamine (10^{-5} M) or isopro-

Table 1. Effects of paraquat (PQ) on cyclic nucleotide levels in rat lung slices plus medium at different times after incubation

	Cyclic nucleotides levels in lung slices plus medium (pmoles/mg protein)							
	2 min		4 min		6 min		12 min	
Addition	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
KRBB (control)	138.56± 6.82 (4)*	4.80±0.62 (4)	133.72±6.78 (4)	4.10±0.32 (4)	148.71± 9.71 (3)	6.33±1.55 (3)	135.02±6.84 (3)	6.37 (2)
PQ (10^{-4} M)	142.04±10.99 (4)	5.07±0.74 (4)	149.10±4.60 (4)	5.67±0.56 (4)	132.53±10.92 (3)	4.93±0.91 (3)	134.06±1.11 (3)	4.15 (2)

* The figures in parentheses are the numbers of slices used.

Table 2. Effects of paraquat (PQ) on histamine and isoproterenol induced changes of cyclic nucleotides in rat lung slices at different times following the incubation

	Cyclic nucleotide levels in lung slices plus medium (pmoles/mg protein)*					
	2 min		4 min		10 min	
	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
Histamine						
KRBB (control)	114.28	6.82	131.96	6.65	128.57	5.76
Histamine (10^{-5} m)	152.77	8.21	161.78	7.14	118.74	6.22
PQ (10^{-4} M)+ Histamine (10^{-5} M)	115.65	4.11	127.27	4.39	131.22	5.05
Isoproterenol						
KRBB (control)	179.81	9.46	158.09	8.82	160.03	11.22
Isoproterenol (10^{-5} M)	317.80	9.60	232.57	7.14	244.49	6.76
PQ (10^{-4} M)+ Isoproterenol (10^{-5} M)	232.75	5.89	223.33	5.02	243.13	5.61

* Each value is the mean of 2 slices.

terenol (10^{-5} M). The incubation was terminated by 1 ml of 25% TCA (cold) followed by homogenization of tissue plus medium with a Brinkman Polytron. After centrifugation, the precipitate was dissolved in 0.2 N NaOH and the protein content determined using bovine serum albumin as a standard[9]. The supernatant fraction was used to determine cAMP and cGMP by the radioimmunoassay method of Frandsen and Krishna[10].

Results and discussion. The effects of paraquat ion (10^{-4} M) on cAMP and cGMP, levels of lung slices at varying times following incubation are shown in table 1. Paraquat in general had no effect on cAMP and cGMP levels of lung slices at anytime except that a slight reduction in cGMP level was noted at 6 and 12 min.

The effects of paraquat (10^{-4} M) on histamine and isoproterenol induced changes in cyclic nucleotides in the lung slices are summarized in table 2. Incubation of lung slices with histamine (10^{-5} M) was found to increase the cAMP level by 34 and 23% at 2 and 4 min respectively and the cGMP level by 20% at 2 min. The finding in the present study that incubation of lung slices with histamine increased the cAMP level is consistent with the finding of other investigators who have previously reported an increased cAMP level in rat lung slices following treatment with histamine[6]. The interesting finding in the present study was that the presence of paraquat (10^{-4} M) in the incubation mixture abolished the histamine mediated elevation of cAMP level. In case of cGMP, paraquat, not only abolished the histamine mediated slight elevation, it caused a further reduction in cGMP level. Consequently, the cGMP level in lung slices treated with paraquat (10^{-4} M) and histamine (10^{-5} M) at 2 and 4 min following the incubation were 50 and 63% of the levels obtained with histamine treatment alone, respectively.

The adrenergic beta agonist, isoproterenol is shown to raise the cAMP level of lung slices but has no effect on cGMP level[7]. In the present study, isoproterenol produced a similar effect on cyclic nucleotides levels in lung slices. For instance, isoproterenol at 10^{-5} M concentration raised the cAMP level of lung slices by 77, 47 and 52% at 2, 4 and 10 min respectively over that of corresponding controls but has little or no effect on cGMP level at 2 and 4 min. The preincubation of lung slices with paraquat (10^{-4} M) inhibited the cAMP elevating effect of isoproterenol only at 2 min and also caused a reduction in the cGMP level by 50% (table 2). How this inhibitory effect of paraquat against the histamine and isoproterenol induced changes of the cyclic nucleotide levels in the lung relates to pathophysiology of this lung toxicant is not understood.

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Ouabain potentiation of Ca release from fragmented cardiac sarcoplasmic reticulum from isolated cat heart

S. Fujino, T. Igarashi, K. Hoshi and M. Fujino*

Department of Pharmacology, Hokkaido Institute of Pharmaceutical Sciences, 62 Katsuraoka-cho 1–7, Otaru 047-02 (Japan), 15 December 1978

Summary. The present study was performed to determine the effect of ouabain on Ca release from fragmented sarcoplasmic reticulum (FSR) isolated from cat cardiac muscles. The results clearly demonstrate that ouabain potentiates the Ca release from FSR by changing the ionic environment.

We have previously reported that microinjection of ouabain into crab muscle fibres produced a marked positive inotropic effect[1]. Furthermore, ouabain potentiated the contractile response in cardiac muscles under the condition of a Ca-free medium prepared by pretreatment with Dowex A-1, Ca-chelete resin[2,3]. These findings suggest that ouabain may produce a increase of Ca release from fragmented sarcoplasmic reticulum (FSR) isolated from cardiac muscles. Recently, it was reported that changing the ionic environment results in a potent release of Ca^{2+} from FSR and the effect is probably due to depolarization of skeletal FSR-membrane[4]. In the present study we tested whether or