Structural alterations in normal and axotomized facial nucleus neurons after treatment with morphine[1]

R.S. Sinatra, L.C. Milks and D.H. Ford

Department of Anatomy and Cell Biology, Downstate Medical Center, Brooklyn (New York 11226, USA), 28 November 1978

Summary. Chronic morphine administration induced ultrastructural alterations in neurons of the facial nucleus and increased the incidence of cell death after axotomy. These findings may correlate with the significant depression of neuronal metabolism known to occur after opiate treatment.

In addition to their specific interactions at regional receptor sites, opiates induce numerous secondary alterations of CNS biochemistry, including pronounced inhibition of RNA and protein synthesis. Moreover, depressions in protein synthesis observed after treatment with morphine were significantly greater in neuronal cell bodies than in surrounding neuropil[2]. Since other pharmacological agents interfering with cellular metabolism markedly influenced the neuronal reaction to axotomy (chromatolysis)[3], it seemed likely that similar alterations would be evident after treatment with opiates. We have recently observed that axotomized facial nucleus neurons were more vulnerable than normal cells to morphine-induced protein synthesis inhibition[4]. This follow-up report will describe alterations of neuronal morphology and regenerative capacity seen after continuous morphine exposure.

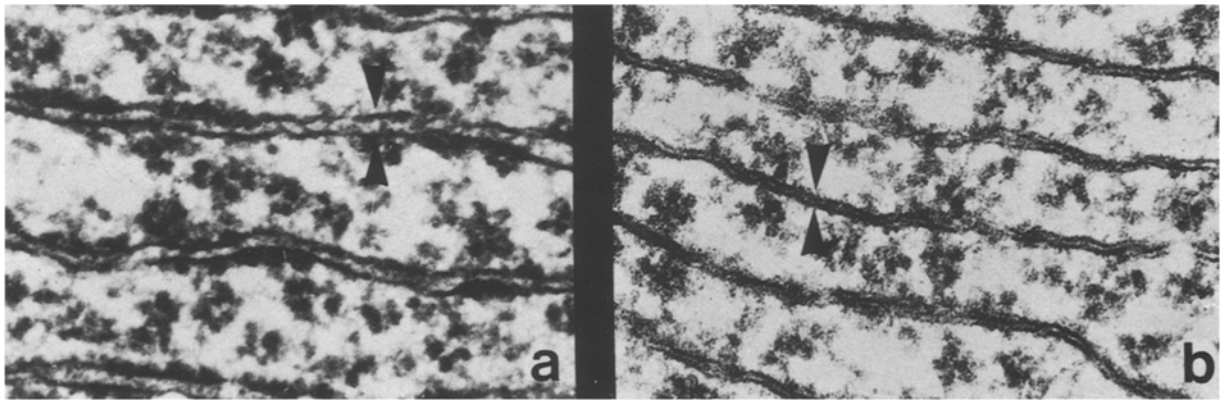
Materials and methods. The left facial nerve of 10 adult male Wistar rats was crushed at its point of emergence deep to the sternocleido-mastoid muscle. The animals were equally divided into 3- and 14-day survival groups (each consisting of 3 drug-treated rats and 2 controls) and were administered morphine sulfate or saline twice daily via an indwelling intrajugular cannula. To eliminate tolerance development (as determined by reaction to a thermal stimulus), an initial 20 mg/kg morphine dose was raised daily to 30 and 40 mg/kg for the 3-day group and to 30, 40, 50, 70, 90, 110 mg/kg for the 14-day animals. The 14-day group was maintained at 110 mg/kg during the 7 days prior to sacrifice. Animals were perfused through the left ventricle with a 1.7% glutaraldehyde–2.5% paraformaldehyde solution. Motor nuclei of the facial nerve were post-fixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in epon-araldite. 1 µm thick plastic sections were cut, stained with toluidine blue and examined by light microscopy. Ultra-thin sections were stained with methanolic uranyl acetate-lead citrate and examined with a Philips 300 electron microscope. In all animals, the facial nucleus on the right side served as the normal (unoperated) control. The ultrastructural characteristics of 1000 unoperated and axotomized neurons (cut through the plane of the nucleolus) were recorded and quantified.

Results. Subtle Nissl body alterations, characterized by a decreased width of rough endoplasmic reticulum (RER) cisternal luminae and loss of membrane-bound ribosomes, were observed in unoperated facial neurons after 3 and 14 days of continuous morphine exposure. Normally, the luminal space of RER cisternae measured 30–60 nm (figure a); in morphinized animals, however, cisternal luminae were often absent and, when present, measured 5–15 nm (figure 1b). Such altered cisternae were found in 70% of the morphine-treated and less than 10% of control neurons. In the affected cells, the range of altered RER profiles varied from 20–80% of the total present within a single neuronal section.

3-days post-axotomy. After continuous morphine exposure, the prominent nucleolar enlargement observed in saline-treated axotomized neurons was not evident. Instead, nucleoli were unusually dark and contained light vacuolated regions. Most of the Nissl bodies exhibited component dispersal (chromatolysis) similar to that observed in untreated axotomized cells. However, flattened RER cisternae with membranes devoid of ribosomes were seen in 55% of morphine-treated cells. Morphine-treated cells contained increased number of lysosomal dense bodies.

14-days post-axotomy. At this survival interval, the appearance of morphine-treated axotomized neurons was similar to that of saline controls, with 2 exceptions. The nucleoli were darkened and rarely enlarged, and a smaller number of neurons had perinuclear concentrations of polysomes (10% of total vs. 30% in saline-treated animals). Chromatolysis and disorganization of RER was comparable to that observed in controls. Flattened cisternae were not seen. Increased numbers of degenerating neuronal profiles were observed in morphine-treated animals (14% of total axotomized cells vs. 5% in saline controls). These neurons, characterized by dark, shrunken perikarya and crenated nuclei were associated with numerous microglial-like cells.

Discussion. Although continuous morphine exposure did not interfere with the onset or magnitude of Nissl dispersal (chromatolysis), it did alter other aspects of the neuronal regenerative response. The absence of nucleolar enlargement and reductions of peri-nuclear ribosomes suggest



a Normal Nissl body from a saline-treated unoperated facial nucleus neuron. Note width of cisternal lumen and membrane-bound ribosomes. × 68,900. *b* Nissl body from an unoperated facial nucleus neuron treated with morphine (3-day administration) demonstrating altered RER cisternae. × 68,900.

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.