increased efferent sympathetic outflow, there could be some other factors involved in cimetidine-induced rise in blood pressure. It has been demonstrated that $H_2$-receptor antagonists metiamide and burimamide possess catecholamine-releasing properties in the periphery (Ganellin & Owen 1977). Furthermore, systemic administration of 6-OHDA does not destroy the chromaffin tissues of the adrenal medulla and the release of catecholamines remains intact (White et al 1979). These observations led us to study the participation of the adrenal medulla in presor action of cimetidine. Bilateral adrenalectomy was performed 3–4 days before the administration of cimetidine. In adrenalectomized SH rats, pressor response of cimetidine given i.c.v. was significantly reduced compared with controls (Table 1), suggesting the participation of the adrenal medulla in cimetidine-induced rise in blood pressure.

Our findings indicate that pressor action of i.c.v. administration of cimetidine is mediated through central catecholaminergic pathways and is due to an increase in efferent sympathetic outflow and release of catecholamines from the adrenal medulla.

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# Effect of morphine on the tissue cyclic AMP and cyclic GMP content in two strains of mice

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The effect of morphine on the cyclic (c) AMP and cyclic (c) GMP concentrations in several organs, and its reversal by naloxone have been investigated in C57BL and DBA strains of mice. Morphine increased the cAMP contents in lungs and muscle, and the cGMP contents in lungs, intestine, heart, liver and muscle in a naloxone-reversible way in C57BL mice only. This is consistent with our previous observation that morphine increased plasma cyclic nucleotide levels in C57BL mice, whereas such an increase was marginal in the DBA strain. These results show that there is a strain difference in the effect of morphine on tissue cyclic nucleotide contents and the possible origin of the plasma cyclic nucleotides which are increased by morphine.

In previous studies, we showed that opioids increase plasma cyclic (c) AMP and cyclic (c) GMP in the ddY strain of male mice (Muraki et al 1979, 1983). We examined the mechanism by which morphine raised plasma cAMP and cGMP concentrations, and suggested that the increase is the result of the morphine-induced activation of the sympathetic and parasympathetic nervous system; the origin of the plasma cyclic nucleotides was assumed to be the peripheral organs but not the central nervous system. Wehmann et al (1974) suggested that the lungs and the small intestine are the site of production of both cAMP and cGMP in dogs, whereas Strange & Mjøs (1975) showed liver to be the major source of the glucagon-stimulated increase in plasma cAMP concentrations.

It is well-known that there is a marked strain difference in the reactivity to opioids (Brase et al 1977; Oliverio et al 1983). The C57BL/6(C57) and DBA/2(DBA) strains of mice especially show many contrasting responses to opioids (Trabucchi et al 1976; Horowitz et al 1977; Frigeni et al 1978, 1981). The administration of opioids increases the locomotor activity in C57 mice, but decreases it in DBA mice, which are more sensitive to the analgesic effect of opioids than are C57 mice (Oliverio & Castellano 1974). We reported that morphine increased plasma cAMP and cGMP levels in C57 mice, whereas the increase was negligible in the DBA strain (Muraki et al 1982).

The purpose of the present study has been to examine the effect of morphine on the cyclic nucleotide concentrations in selected organs of two strains of mice, C57 and DBA, to determine the possible sources of the

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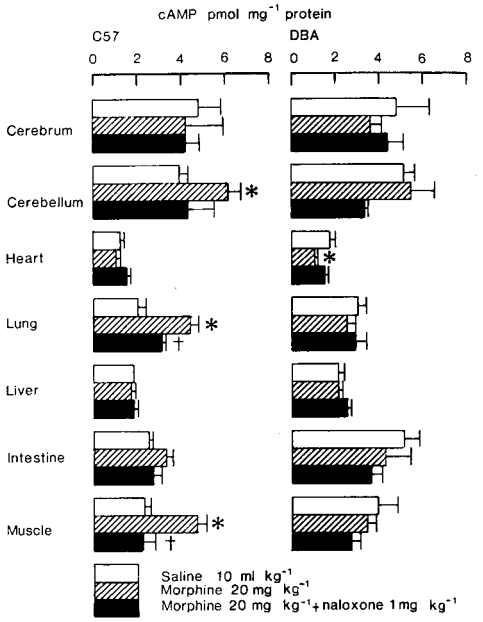


FIG. 1. Difference between C57 and DBA strain mice in the effect of morphine and naloxone on tissue cAMP content. Tissue cAMP was determined 30 min after the s.c. administration of drugs. Open columns, saline (10 ml $kg^{-1}$); hatched columns, morphine (20 mg $kg^{-1}$); shaded columns, morphine (20 mg $min^{-1}$) + naloxone (1 mg $kg^{-1}$). Each column represents mean cAMP content (pmol $mg^{-1}$ protein) ± s.e. of 6 mice. Statistical significance in Student's *t*-test: $^{*}P < 0.05$ vs saline; † $P < 0.05$ vs morphine.

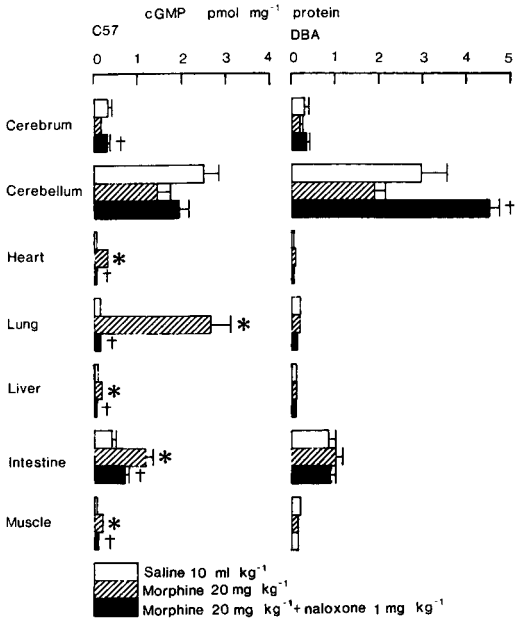


FIG. 2. Difference between C57 and DBA strain mice in the effect of morphine and naloxone on tissue cGMP content. Tissue cGMP was determined 30 min after the s.c. drug administration. Each column represents mean cGMP content (pmol $mg^{-1}$ protein) ± s.e. of 6 mice. For details, see the legend to Fig. 1.

plasma cyclic nucleotides, and whether there is a strain difference in the effect of morphine on tissue cyclic nucleotides in the two strains.

*Methods*

Adult male mice of C57BL/6N(C57) and DBA/2N(DBA) strains, obtained from Charles River Japan, Inc. (Atsugi, Japan), were given 0·9% NaCl (saline) (10 ml $kg^{-1}$), morphine HCl (20 mg $kg^{-1}$) or both morphine HCl (20 mg $kg^{-1}$) and naloxone HCl (1 mg $kg^{-1}$) subcutaneously. Thirty minutes later, when the peak effect of morphine was known to increase plasma cyclic nucleotide concentrations (Muraki et al 1979, 1983), the mice were exposed to whole-body microwave irradiation (Toshiba Microwave Applicator TMW 6402A) (Moroji et al 1977) (4 kW, 1·3 s) to inactivate enzymes. Cerebrum, cerebellum, lungs, heart, liver, ileum and muscle (M. biceps femoris) were quickly dissected, frozen with liquid nitrogen and stored at −70 °C until use. The weighed tissue (200–30 mg) was homogenized in 2 ml 0·1 M HCl and the homogenate centrifuged at 1500*g* for 5 min. The supernatant was succinylated with succinic anhydride and was directly assayed for cAMP and cGMP by radioimmunoassay (Honma et al 1977). Protein was determined by the method of Lowry et al (1951). Morphine HCl was purchased from Sankyo Co. Ltd (Tokyo), cAMP and cGMP radioimmunoassay kits from Yamasa Shoyu Co., Ltd (Choshi, Japan). Naloxone HCl was a gift from Endo Labs Inc. (Garden City, NY). Other drugs were obtained from commercial sources. The results were evaluated statistically by Student's *t*-test.

*Results*

There was no strain difference in the tissue cAMP of the saline control mice except in the intestine, where DBA mice showed the higher cAMP concentration ($P < 0.01$) (Fig. 1). Only in the liver and muscle did the cGMP concentrations of the saline-treated mice differ, the DBA strain mice values were higher ($P < 0.01$) (Fig. 2).

Morphine increased cAMP in the cerebellum, lungs and muscle of C57 mice and the increase elicited by morphine, except in the cerebellum, was antagonized by co-administration of naloxone. In the DBA strain, morphine did not increase the cAMP of the organs. The cAMP content of the heart in DBA mice was slightly decreased by morphine but its reversal by naloxone was not significant.

In C57 mice, morphine increased the cGMP content of heart, lungs, liver, intestine and muscle in a naloxone-reversible way, whereas the tissue cGMP content in DBA mice was not changed by morphine. The cerebellar cGMP levels of both C57 and DBA mice

were not significantly affected by morphine, although the cerebellar cGMP in DBA mice treated with both morphine and naloxone was higher than that of DBA mice treated with morphine alone.

*Discussion*

The results observed with saline-treated mice of both strains indicate that the basal cyclic nucleotide contents of the organs examined did not differ, with some exceptions: the cAMP content of the intestine and cGMP content of liver and muscle of DBA strain mice was higher than that of the C57 strain; the cause of the difference is not clear.

Morphine increased tissue cyclic nucleotide levels in several organs of C57 mice but not of the DBA strain. This strain difference in the effect of morphine on the tissue cyclic nucleotide content in the mice is consistent with our previous observation that morphine increased the plasma cyclic nucleotide level in the C57 strain but only slightly changed it in the DBA strain (Muraki et al 1982). The increase in the tissue cyclic nucleotide content in C57 mice treated with morphine was antagonized by naloxone with the sole exception of cAMP in the cerebellum, indicating the involvement of the opiate receptor. Morphine increased the cAMP content in the cerebellum, lungs and muscle, and the cGMP content of the heart, lungs, liver, intestine and muscle, suggesting that lungs and muscle may be the source of the morphine-induced increase in the plasma cAMP, and that lungs and intestine may be the source of the morphine-induced increase in plasma cGMP in C57 mice. Since the cGMP content in the heart, liver and muscle of the morphine-treated mice is less than that found in the lungs and intestine, the contribution of the former organs to increase the overall plasma cGMP level would be small.

We found that morphine increased the cerebellar cAMP content in the C57 but not in the DBA strain, whereas Racagni et al (1979) reported no change in cAMP concentrations in the C57 strain. The authors also reported morphine to increase cGMP in the cerebellum of C57 mice, whereas it was decreased in the DBA strain when determined 10 min after 10 mg $kg^{-1}$ morphine i.p. In the present study, morphine did not significantly change the cerebellar cGMP content in either the C57 or DBA strain. The increase in cerebellar cGMP after co-administration of morphine and naloxone above that of morphine alone in DBA mice, might not be related to the opioid effect, because morphine alone did not significantly decrease the cGMP levels in DBA mice. At present we have no good explanation for the discrepancy, however, it may be due to the different times after morphine injection when the cerebellar cGMP was examined.

In the present study, we did not examine the cause of the difference between C57 and DBA, but we have previously suggested that the difference in the plasma cyclic nucleotide response to morphine may be mainly due to the difference in the central opioid receptors, rather than to the response of adenylate cyclase of the guanylate cyclase system to stimulation of β-adrenoceptors or muscarinic receptors (Muraki et al 1982).

In conclusion, we have demonstrated that there is a strain difference in the effects of morphine on the tissue cyclic nucleotide concentrations between C57 and DBA mice. In accordance with the changes in plasma cyclic nucleotide level, the morphine-induced increase in the tissue cyclic nucleotide level was seen in the C57 mice but not in the DBA strain, and the increase was reversed by naloxone, suggesting the involvement of the opioid receptors. Our results indicate that the major sources of morphine-induced increase in plasma cyclic nucleotides in C57 mice are lungs and muscle for cAMP, and lungs and intestine for cGMP.

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