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Developmental responses to opioids reveals a lack of effect on stress-induced corticosterone levels in neonatal rats

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1 The neonate has an unusual capacity for survival and the possibility exists that mechanisms for controlling stress responses may differ in the developing animal. In adults both endogenous and exogenous opioids can modulate the corticosterone responses to stress. We have studied this effect in neonatal rats and found that opioid modulation is absent in early postnatal development.

2 Neonatal rats of either sex were injected with morphine (5–50 mg kg^{-1}), fentanyl (10–100 μg kg^{-1}), buprenorphine (0.1–30 mg kg^{-1}) or naloxone (0.1–10 mg kg^{-1}) and plasma corticosterone measured fluorimetrically 15 or 20 min later. In addition naloxone reversibility studies (1 mg kg^{-1}, co-administered) were carried out for the opioid agonists.

3 In adult rats, elevations in plasma corticosterone caused by injection stress were potentiated by morphine, fentanyl and buprenorphine. In neonates, though injection stress-induced rises in plasma corticosterone were absent at 10 days, elevations were observed at 21 days and later. However, significant potentiation of this corticosterone response by fentanyl was absent at 21 days and at later ages (30 and 40 days) for morphine and buprenorphine. The potentiating effect of all three agonists did not become fully effective until day 45. In addition, in animals acclimatized to injection stress by 7 day injection pretreatment, fentanyl did not significantly alter corticosterone levels in 30 day old neonates.

4 High doses of naloxone (10 mg kg^{-1}) significantly increased the corticosterone response to injection stress in adult rats but this effect was absent in 30 day old animals. A dose of naloxone (1 mg kg^{-1}) which had no significant effect on the corticosterone response inhibited the effects of morphine, fentanyl and buprenorphine in 45 day old and adult rats.

5 This late development of opioid action is unusual in comparison with the maturation of endogenous peptides, receptors and antinociceptive responses and suggests that alternative mechanisms may be involved in stress-control in the neonate.

Introduction

Endogenous opioid peptides appear to be involved in the control of the hypothalamus-pituitary-adrenal system. In particular there is evidence that stress responses are modulated by opioids. For example footshock alters levels of plasma β-endorphin and hypothalamic [Leu] enkephalin (Rossier *et al.*, 1977; 1978) and adrenalectomy lowers the content of hypothalamic opioid peptides (Gibson *et al.*, 1980). In addition the basal and stress-induced secretion of corticosterone in the rat have been shown by a number of laboratories to be modulated by the acute administration of opioid agonists (Kokka *et al.*, 1973; Eisenberg, 1985; Pechnick *et al.*, 1985a) and opioid antagonists (Eisenberg, 1980; Siegel *et al.*, 1982; Jezova *et al.*, 1982).

Stress responses in neonatal rats appear during the third postnatal week (Haltmeyer *et al.*, 1966; Kakihana *et al.*, 1974), but there are no studies in the literature pertaining to opioid control of stress responses during development. Accordingly we have studied the ontogeny of opioid modulation of corticosterone responses to stress from day 10 to adulthood.

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Methods

Animals and experimental conditions

Wistar albino rats of either sex were used in all experiments and maintained in a constant 12 h light cycle environment (lights on at 07 h 00 min). Twenty-four hours before experimentation the animals were equilibrated in a quiet laboratory where all procedures were carried out. Neonates were cross-fostered at birth and weaned at day 20. In experiments on 10 day old rats the mother was removed immediately before drug injection. On each experimental day basal and saline-injected controls were determined in parallel with measurements after administration of opioid drugs. The sequence of drug injections was randomized on a daily basis.

Each mean determination represents equal numbers of males and females taken from at least three litters. In addition measurements were made on at least three separate days. All experimental manipulations were carried out between 08 h 30 min and 09 h 30 min.

Drug administration and corticosterone assay

Morphine sulphate (Macfarlan Smith), fentanyl citrate (Janssen Pharmaceuticals), buprenorphine hydrochloride (Reckitt and Colman), naloxone hydrochloride (Dupont) and 0.9% w/v NaCl solution (saline) were injected intraperitoneally in a volume of 0.1–0.2 ml. Concentrations are expressed as salts. In one series of experiments animals were acclimatized to injection either by twice daily injections of saline (at 09 h 00 min and 17 h 00 min) or three daily injections (at 09 h 00 min, 13 h 00 min and 17 h 00 min) for 7 days before experimentation. For naloxone reversibility studies morphine, fentanyl or buprenorphine were administered concomitantly with naloxone (1 mg kg^{-1}). Saline plus naloxone controls were determined in parallel experiments. Rats were killed by decapitation and trunk blood was collected into heparinized tubes 15 min after injection for fentanyl and naloxone and 20 min after injection for morphine and buprenorphine. Plasma was prepared and corticosterone determined fluorimetrically as described previously (Kitchen & Rowan, 1984). Standard curves for corticosterone were prepared in duplicate for each experiment.

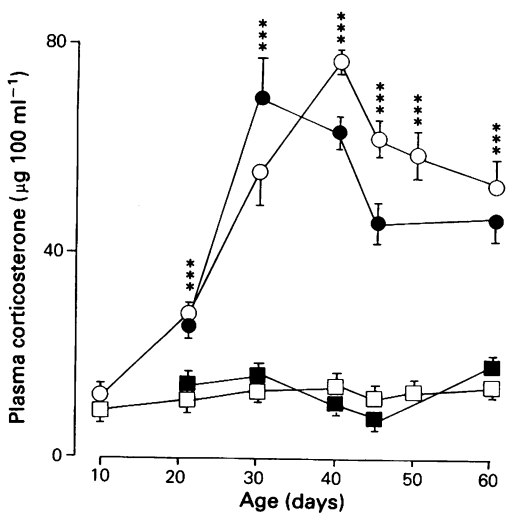

Figure 1 Basal and stress-induced plasma corticosterone levels in the developing rat. Points represent mean of at least 6 observations and vertical lines indicate s.e.mean. (□, ■) Basal levels in naïve animals as controls for 15 and 20 min studies, respectively. (○, ●) Levels 15 and 20 min after i.p. injection of 0.9% saline, respectively. ***$P < 0.001$, compared to basal level (Student's t test).

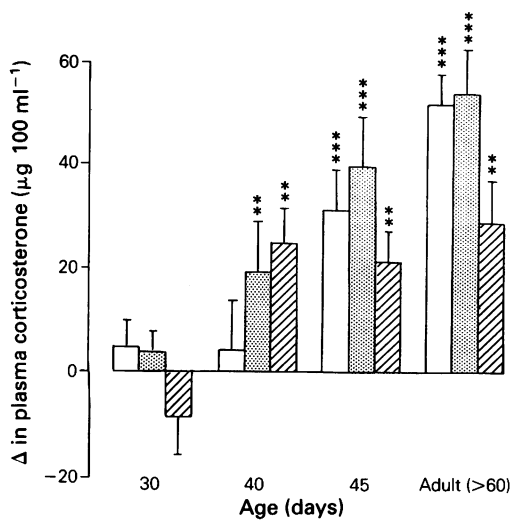

Figure 2 Effect of acute morphine administration on plasma corticosterone levels in the developing rat. Values represent a change in plasma corticosterone from saline-injected controls. Each column shows the mean of at least 6 observations and vertical lines indicate s.e.mean. Open columns, 5 mg kg^{-1} morphine sulphate; stippled columns, 10 mg kg^{-1} morphine sulphate; hatched columns 50 mg kg^{-1} morphine sulphate. **$P < 0.01$; ***$P < 0.001$, vs. saline-injected controls (t test, non transformed data).

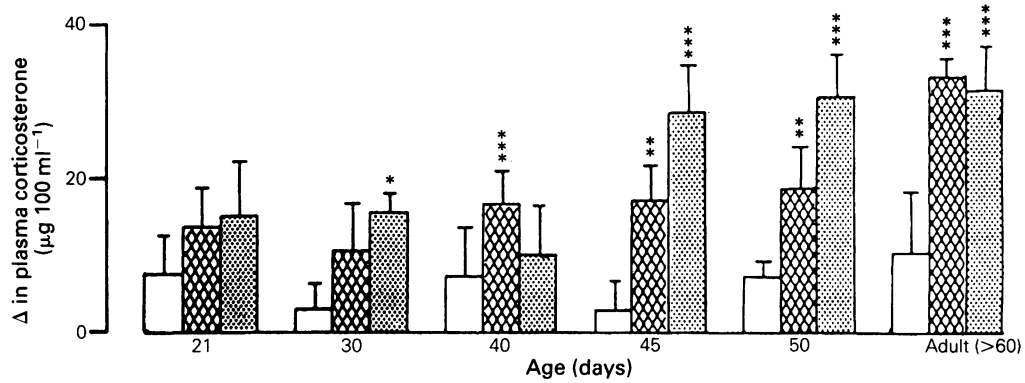

Figure 3 Effect of acute fentanyl administration on plasma corticosterone levels in the developing rat. Values represent a change in plasma corticosterone from saline-injected controls. Each column shows the mean of at least 6 observations and vertical lines indicate s.e.mean. Open columns, $10\,\mu g\,kg^{-1}$ fentanyl citrate; cross-hatched columns, $50\,\mu g\,kg^{-1}$ fentanyl citrate; stippled columns, $100\,\mu g\,kg^{-1}$ fentanyl citrate. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$, vs. saline-injected controls (t test, non-transformed data).

Results

Ontogeny of basal and stressed corticosterone levels

Basal levels of corticosterone were unaltered during postnatal development (10–50 days, Figure 1). The development of plasma corticosterone responses to injection stress is shown in Figure 1. Stress-induced rises were absent in 10 day rat pups but significant elevations were observed at 21 days. The magnitude of this stress-induced rise increased with age and peaked at around postnatal day 40. Stress-induced elevations at later ages were lower, adult responses appearing by 45 days.

Ontogeny of opioid effects on corticosterone

In adult rats elevations in plasma corticosterone caused by injection stress were potentiated by morphine, fentanyl and buprenorphine (Figures 2, 3 and 4). The magnitude of the potentiation by fentanyl exhibited a maximal increment of 63%. Morphine produced a more marked potentiation (up to 114%) but at high doses ($50\,mg\,kg^{-1}$) the potentiating effect was reduced. Buprenorphine also produced a marked potentiation (up to 89%) at doses above $0.3\,mg\,kg^{-1}$; this effect was not dose-related.

In 30 day old rats the potentiating effect of morphine was virtually absent (Figure 2). Marked and significant elevations in corticosterone above those in saline injected controls were not observed until day 40 and adult responses attained by day 45. Responses to fentanyl were also markedly less in neonatal animals

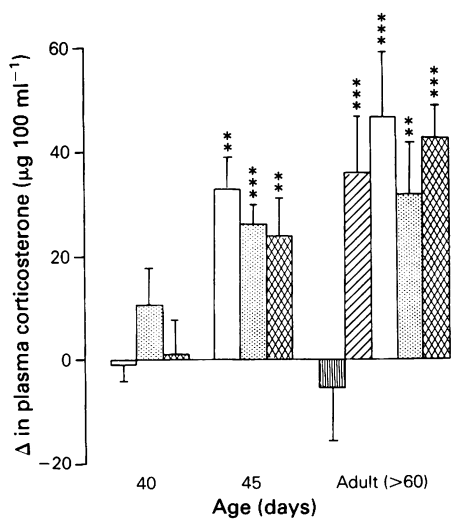

Figure 4 Effect of acute buprenorphine administration on plasma corticosterone levels in the developing rat. Values represent a change in plasma corticosterone from saline-injected controls. Each column shows the mean of at least 6 observations and vertical lines indicate s.e.mean. Vertically-hatched columns $0.1\,mg\,kg^{-1}$; diagonally-hatched columns, $0.3\,mg\,kg^{-1}$; open columns, $1\,mg\,kg^{-1}$; stippled columns, $3\,mg\,kg^{-1}$; cross-hatched columns, $30\,mg\,kg^{-1}$ buprenorphine hydrochloride. $^{**}P<0.01$; $^{***}P<0.001$, vs. saline-injected controls (t test, non-transformed data).

though a small potentiating effect could be demonstrated in 21, 30 and 40 day old rats. Responses to buprenorphine were absent at 40 days and significant potentiating activity was not observed until day 45.

In 30 day old animals acclimatized to injection-stress by 7 days' prior injection, plasma corticosterone levels after saline administration were not so markedly elevated but still significantly higher than basal levels (Table 1). In these animals fentanyl did not further elevate plasma corticosterone at any of the dose levels tested.

Administration of naloxone (0.1–1.0 mg kg^{-1}) failed to alter plasma corticosterone levels in 21 and 30 day neonatal rats, but in adult animals 10 mg kg^{-1} produced a significant potentiation of corticosterone levels (Figure 5).

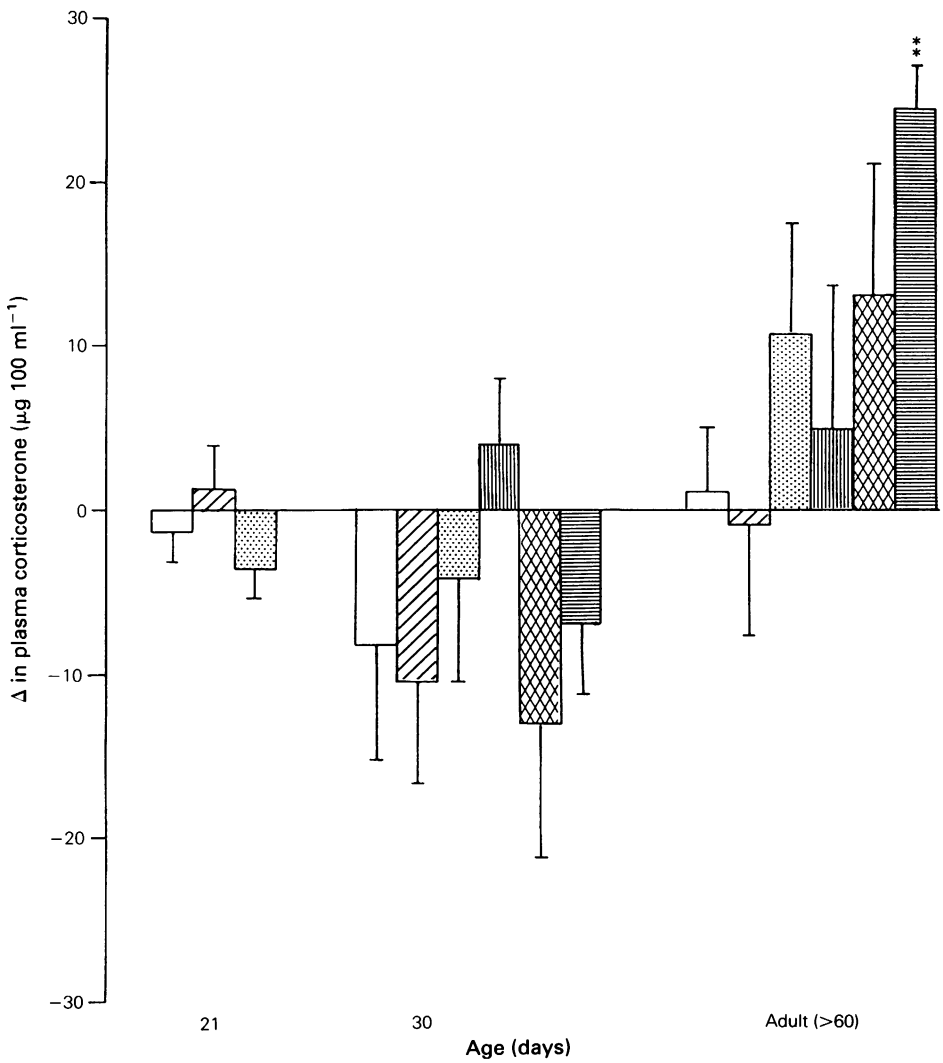

Figure 5 Effect of acute naloxone administration on plasma corticosterone levels in the developing rat. Values represent a change in plasma corticosterone from saline-injected controls. Each column shows the mean of at least 6 observations and vertical lines indicate s.e.mean. Open columns 0.1 mg kg^{-1}; diagonally-hatched columns, 0.5 mg kg^{-1}, stippled columns, 1.0 mg kg^{-1}; vertically-hatched columns, 5.0 mg kg^{-1}; cross-hatched columns 7.5 mg kg^{-1}; horizontally-hatched columns, 10 mg kg^{-1} naloxone hydrochloride. $^{**}P < 0.01$, vs. saline-injected controls (t test, non-transformed data).

Table 1 Plasma corticosterone ($\mu g\,100\,ml^{-1}$) 15 min after injection of fentanyl in 30 day old rats previously acclimatized to injection stress

Treatment		*Injection protocol* 7 days b.d.	7 days t.d.s.
Basal		13.1 ± 1.6	10.6 ± 0.6
Saline		36.0 ± 5.6	32.1 ± 4.8
Fentanyl	$10\,\mu g\,kg^{-1}$	39.8 ± 3.6	35.0 ± 7.7
	$50\,\mu g\,kg^{-1}$	47.9 ± 2.7	41.4 ± 6.7
	$100\,\mu g\,kg^{-1}$	44.2 ± 5.5	44.3 ± 5.1

Values are mean ± s.e.mean of 6 observations.
Data with fentanyl not significantly different from those with saline (Student's *t* test).

Concomitant administration of a dose of naloxone ($1\,mg\,kg^{-1}$) in 45 day and adult animals which failed to alter significantly plasma corticosterone reduced the potentiating effect of all three opioid agonists. In 45 day old rats the maximal % increase in the presence of naloxone was 21, 26 and 11% for morphine, fentanyl and buprenorphine, respectively. This compares with maximal increments of 86, 46 and 63% for these opioids alone. In adults, the maximal % increase induced by the opioid agonists was also markedly reduced with concomitant administration of naloxone (38, 10 and 22% vs. 114, 63 and 89% for morphine, fentanyl and buprenorphine). Only one dose of fentanyl significantly increased corticosterone levels in the presence of naloxone (Figure 6).

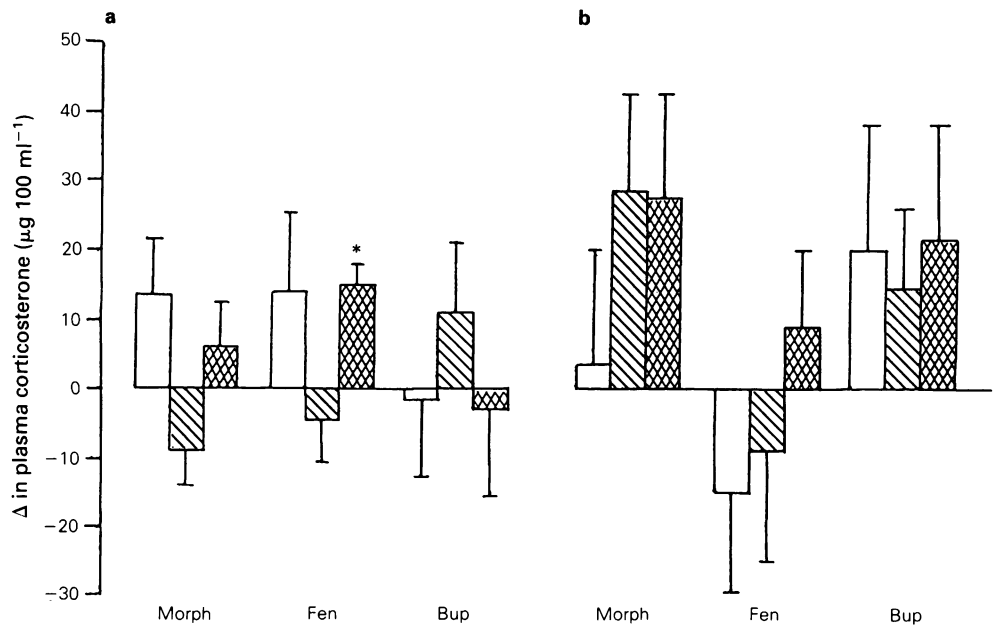

Figure 6 Effect of naloxone on change in plasma corticosterone elicited by morphine (Morph), fentanyl (Fen) and buprenorphine (Bup) in (a) 45 day old and (b) adult (> 60 day) rats. Values represent a change in plasma corticosterone from naloxone plus saline-injected controls. Open columns, $5\,mg\,kg^{-1}$ morphine sulphate, $10\,\mu g\,kg^{-1}$ fentanyl citrate or $1\,mg\,kg^{-1}$ buprenorphine hydrochloride; diagonally-hatched columns, $10\,mg\,kg^{-1}$ morphine sulphate, $50\,\mu g\,kg^{-1}$ fentanyl citrate or $3\,mg\,kg^{-1}$ buprenorphine hydrochloride; cross-hatched columns $50\,mg\,kg^{-1}$ morphine sulphate, $100\,\mu g\,kg^{-1}$ fentanyl citrate or $30\,mg\,kg^{-1}$ buprenorphine hydrochloride. $^*P < 0.05$, vs. naloxone plus saline-injected controls (*t* test, non-transformed data).

Discussion

Basal corticosterone levels were constant between postnatal days 10 and 50 which accords with the results of Allen & Kendall (1967) and Kakihana *et al.* (1974), but is in contrast to those of Henning (1978) and Walker *et al.* (1986) who found a marked rise in basal levels between the second and third postnatal week. The absence of stress-induced rises in corticosterone due to saline injection at day 10 confirms results with other stressors where adrenal responses are not apparent at this age (Haltmeyer *et al.*, 1966; Henning, 1978), although recently rises in corticosterone due to ether, electroshock or hypoxia have been demonstrated in 10 day old rat pups (Walker *et al.*, 1986). Also in common with most forms of stress activation (Haltmeyer *et al.*, 1966; Allen & Kendall, 1967; Kakihana *et al.*, 1974), injection causes marked elevations by postnatal day 21. The peak in stress responses at day 40 suggests a late postnatal sensitivity, and a delay in maturation of hypothalamus-pituitary function. Interestingly, circadian rhythms of corticosterone are not fully developed until day 40 (Allen & Kendall, 1967).

There is disagreement in the literature over the effects of opioid agonists and antagonists on stress-induced changes in corticosterone. The effect appears to be dependent upon species, dosage, time course of treatment and stressor (Gibson *et al.*, 1979; Siegel *et al.*, 1982; Kitchen & Rowan, 1984). We observed potentiation of injection stress responses in adult rats by morphine, fentanyl and buprenorphine at antinociceptive doses. The magnitude of potentiation by morphine is similar to that observed in rats acclimatized to handling and injection responses (Pechnick *et al.*, 1985a). Gibson *et al.* (1979) have demonstrated that morphine behaves as an antagonist of normorphine potentiation of ether-stress responses in mice. Although morphine showed a biphasic dose-response effect in our study this does not appear to be consistent with partial agonism of this opioid,since morphine exhibited a greater maximum effect than the full agonist fentanyl. In addition it should be noted that buprenorphine, recognized to exhibit partial agonist activity in antinociceptive tests (Cowan *et al.*, 1977), did not behave like morphine in its effects upon corticosterone.

It is clear that the modulating activity of opioid agonists upon corticosterone is absent or at least severely reduced in neonates up to around day 45. The possibility that this was due to a ceiling effect on the rise in corticosterone due to injection was investigated by attempting to acclimatize neonates by repeated injection 7 days before experimentation. This manipulation is successful in abolishing injection stress responses in adult rats (Hayes & Stewart, 1985) but in our study was only partially successful in neonates. Nevertheless there was little indication of opioid modulation in neonates where stress-induced rises were limited by the pre-experimental gentling procedure (Table 1). There is some indication of differential time courses for the three opioids studied and fentanyl effects appeared slightly earlier than those of morphine or buprenorphine. The reasons underlying this are not clear but are unlikely to be receptor related since all three opioids are μ-agonists.

Naloxone reversibility studies confirmed that the opioid agonist effects upon corticosterone are mediated by opioid receptors, in both adult animals and at the earliest age where this response appears (45 days). This accords with studies in other laboratories in the adult rat (Pechnick *et al.*, 1985b).

Naloxone has been found by some laboratories to resemble opioid agonists in its effects upon corticosterone (Eisenberg, 1980; Siegel *et al.*, 1982). We only observed significant potentiation of corticosterone responses at high doses; doses considered to be more representative of selective opioid receptor antagonism were without effect. This accords with evidence from other laboratories (Tapp *et al.*, 1981; Pechnick *et al.*, 1985b). There are also species differences since mouse corticosterone levels showed biphasic responses to naloxone in our laboratory under identical experimental conditions to the rat study described here (Kitchen & Rowan, 1984). Since 30 day old neonates showed no response to naloxone, the difference between neonates and adults exhibited by opioid agonists appears to be shared by the opioid antagonist.

The late development of opioid activity on hypothalamus-pituitary-adrenal functioning is unusual in comparison with many physiological effects of opioids (such as analgesia) which parallel the opioid receptor development (Kitchen *et al.*, 1984). μ-Receptor development and CNS peptide levels reach maturity by day 21 (Bailey & Kitchen, 1985; Spain *et al.*, 1985) and even δ-receptors which appear later in postnatal development peak by day 30 (Spain *et al.*, 1985; McDowell & Kitchen, 1986). Thus other factors must influence the control exerted by opioids on the hypothalamus-pituitary-adrenal axis. It is unlikely to be related to immaturity of other intermediary neurochemical systems involved in control of ACTH since, like opioid systems, these develop rapidly in the early postnatal period (Coyle & Enna, 1976; Nomura *et al.*, 1976). It is not possible at this stage to discern the alternative mechanisms which may control stress responses in the developing neonate. Whether this is related to the capacity for survival in the newborn is at present a matter of conjecture.

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