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EFFECT OF OPIOID ANTAGONISTS IN STRESS-INDUCED ANALGESIA IN *UROMASTJX HARDWICKII* AND *GALL US DOMESTICUS*

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ABSTRACT

The effect of a repetitive stress (induced by electric shocks), with/without the administration of different doses [0.125 (a), 0.250 (b) and 0.500 (c) mg/kg body weight of animal] of various opioid antagonists (naloxone hydrochloride (N), pentazocine lactate (P) and butorphanol tartrate (B)], on heart rate/ECG, respiratory rate and core body temperature, were studied in *Uromastic hardiwickii* and *Gallus domesticus.* A significant increase in heart rate of *Uromastix* was observed with Nb, Nc, Pa, Pb, Pc, Ba and Bb, while respiratory rate raised significantly by using Na, Nb, Nc and Bp. A significant increase in rooster's heart rate was noticed with Na, Pa, Ba and Bc. On the other hand, the respiratory rate of rooster significantly enhanced with Na, Nc, Pa, Bb and Bc.

These results suggest that endogenous opioids are involved in the phenomenon of stress-induced analgesia.

Introduction

Morphine and other opioids exert their actions by interacting with specific receptors present within the CNS and in peripheral tissues. The opioid component of stress-induced antinociception, for most stressors, appears to involve mu opioid receptors but three are indication sin both mice (Heart et al., 1985) and rats (Jackson and Kitchen, 1989) that delta opioid receptors may also contribute to this effect. Nalorphine, pentazocine, nalbuphine, butorphanol and naloxone display antagonistic actions on both mu and delta receptors. In the mid 1970s, a number of peptides having morphine-like actions were isolated from mammalian brain, pituitary, spinal cord and gastrointestinal tract (Tripathi, 1988). These are active in very small amounts, their actions are blocked by naloxone, and they bind with high affinity to the opioid receptors. The opioid peptides (endorphins, enkephalins and dynorphins) constitute an endogenous opioid system which normally modulates pain perception, mood, hedonic and motor behavior, emesis, pituitary hormone release, gastrointestinal motility etc. The wide distribution of enkephalins and dynorphins and their short half-lives suggest their function as neurotransmitter. They appear to

regulate pain responsiveness at spinal and supraspinal levels. The response to opioid receptor activation is probably expressed through inhibition of cAMP production in target cells (Tripathi, 1988).

Footshock analgesia and analgesia produced by conditioning to footshock (autoanalgesia) displays nonopioid characteristics in cross-tolerance and opiate receptor antagonist studies (Bodnar, 1990). In contrast, naloxone is found to block analgesia induced by either immobilization (Amir et al., 1980) or by food deprivation (Bodnar et al., 1980). Thus, it becomes apparent that different stressors appear to elicit either opioid-mediated or nonopioid-mediated analgesic responses, suggesting that multiple opioid and nonopioid analgesic systems exist that are differentially activated by different classes of environmental stimuli (Bodnar, 1990).

Present studies were undertaken to observe the effect of a repetitive stress, induced by electric shocks, with or without the administration of different doses of various opioid antagonists, on the following somato-vegetative parameters: heart rate/ECG, respiratory rate and core body temperature in two widely different species of animals: *Uromastic hardwickii* and *Gallus domesticus,* with a view to assess whether endogenous opioids are involved in the phenomenon of antinociception in these animals.

Materials and Methods

Animals Used:

a) *Uromastix hardwickii:* Twenty healthy male *Uromastices* of mean weight 121.40±0.96 g were used in all experiments.
b) *Gallus domesticus* (breed:leghorn): Twenty fully grown (6-8 weeks), healthy chickens (roosters) of mean weight 1.58±0.88 kg were used in all experiments.

The animals were habituated first to the laboratory environment before experiments. To be used for experimentation on the next day, the animal was not fed overnight at all.

Drugs Used:

A) Naloxone hydrochloride (0.4 mg/ml ampules)
Narcan Injection – Du Pont Pharmaceutical, Inc.)
B) Pentazocine lactate (30 mg/mI ampulses)
(Sosegon – Sterling Wintrhrop S.A.)
C) Butorphanoltartrate (1 mg/ml vials)
(Stadol – Bristol Laboratories)

Doses of Drugs Administered:

0.125, 0.250 and 0.500 mg/kg body weight of animal were used. Same doses were administered intramuscularly to both animals.

Apparatus Used:

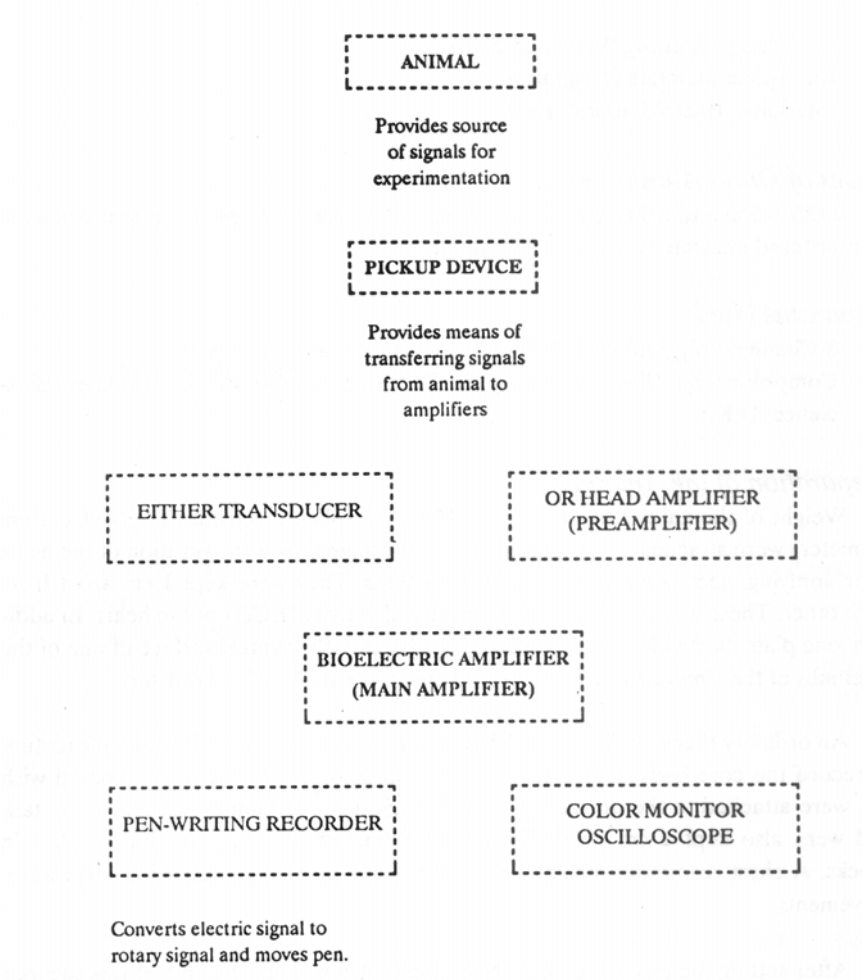
A) 8-Channel Polygraph-363 (NEC San-ei Instruments Ltd., Japan).
B) Comprehensive Electrophysiological Teaching Unit (CEPTU) (Palmer Bioscience, U.K.).

Preparation of the Animal:

Weight of the animal was recorded. Then, two plate electrodes (Ag/AgCl, 1 cm diameter) were attached to the ventral skin of the animal over the position of the heart after applying electrode gel(DRACARD) to them. They were kept 1 cm apart from each other. These electrodes picked up electrical activity (ECG) of the heart. In addition, one plate electrode, coated with gel, was fixed to the ventral surface of one of the forelimbs of the *Uromastix* or to the skin of one of the thighs of the rooster.

An ordinary thermometer with Celsius scale was inserted into the animal's rectum to record the core body temperature. Two stimulating plate electrodes, coated with gel, were attached to the dorsal lower trunk region of the animal just above the tail, and were also kept 1 cm apart. They were intended for the provision of electric shocks. A chest respiration pickup, set over the animal's lungs, recorded respiratory movements.

After setting the electrodes and chest respiratory pickup, the animal was secured tightly, and then allowed to stabilize over the time at the room temperature ($30^{o}\pm30^{o}$C). The amplified signals of the two parameters (ECG and respiration rate) were monitored on a 4-channel color monitor oscilloscope and recorded on an 8-channel thermal pen-writing recorder. The components of the complete recording system were arranged as shown in the following sequence:



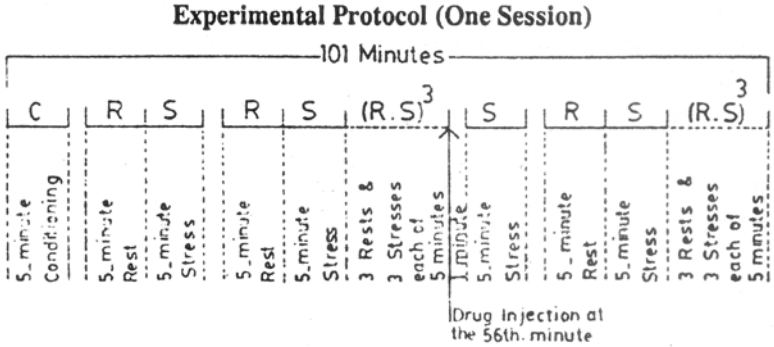
A stimulus isolator was used with the stimulator of the CEPTU. The instrument uses the stimulator pulse output to generate high voltage isolated pulse at controlled low current. A threshold of 1 mA was employed during the "Conditioning Phase", while that of 5mA during all "Stressing Phases" of the experiments.

Recording of the Physiological Parameters

Recording of the normal parameters was carried out for 10 minutes. The stimulator was kept on OFF position because no stress was needed during the normal recordings.

Experimental Protocol:

The experimental procedures of Willer and albe-Fessard (1980) was adapted for the recording of all the physiological parameters.



Where,

(C) Conditioning phase = Duration of recording in which low current of lmA threshold was applied.

(R) Resting phase = Duration where no recording was made an no current was applied.

(S) Stressing phase = Duration of recording in which high current of 5mA threshold was applied.

During a single experimental session, only one dose of each opioid antagonist was used. Three sessions were done in order to complete the experiments for the effects of opioid antagonist under study. In the same way, the whole process was repeated with every other *Uromastix* and rooster. Each animal was subjected to 9 experimental sessions and only one session was done in a single day.

Results

"Student T-test" was applied separately to each set of data obtained. P-value was calculated by making a comparison of the results obtained during the 5 "Stressing Phases" prior to the administration of drug with those obtained during the S "Stressing Phases" following the drug administration.

The following significant ($P<0.05$) changes were observed in *Urontastix* heart and respiration rates after drug administration:

i) Heart rate with 0.125 mg drug/kg body weight of animal

Pentazocine: $51.31\pm0.36 \rightarrow 59.50\pm1.57$

Butorphanol: $46.36\pm1.04 \rightarrow 52.577\pm2.86$

ii) Heart rate with 0.250 mg drug/kg body weight of animal
Naloxone: 62.1711.40 → 70.8320.40
Pentazocine: 61.9221.94 →783221.11
Butorphanol: 61.9020.10 → 74.001032

iii) Heart rate with 0500 mg drug/kg body weight of animal
Naloxone: 58.2925.42 → 7713120.17
Pentazocine: 67.2312.09 → 80.2721.47

iv) Respiration rate with 0.125 mg drug/kg body weight of animal
Naloxone: 14.782032 →19.5011.85

v) Respiration rate with 0.250 mg drug/kg body weight of animal
Naloxone: 19.1710.17 → 34.6712.65
Butorphanol: 18.5811.16 → 22.4510.58

vi) Respiration rate with 0.500 mg drug/kg body weight of animal
Naloxone: 18.53±0.60→ 26.0025.09

The following significant ($P<0.05$) changes were noted in rooster's heart and respiration rates after drug administration.

i) Heart rate with 0125 mg drug/kg body weight of animal
Naloxone: 326.8322.13 → 338.381330
Pentazocine: 308.71t 2.02 → 318.3314.81
Butorphanol: 311.5713.48 → 322.77514.11

ii) Heart rate with 0.250 mg drug/kg body weight of animal
Butorphanol: 323.0021.82 → 335.5015.06

iii) Heart rate with 0.500 mg drug/kg body weight of animal
Butorphanol: 309.7811.35 → 313.8924.61

iv) Respiration rate with 0.125 mg drug/kg body weight of animal
Naloxone: 20.3310.62→ 24.2910.67
Pentazocine: 23.3010.40 → 27.2310.88

v) Respiration rate with 0.250 mg drug/kg body weight of animal
Butorphanol: 26.2510.50 → 33.8221.18

vi) Respiration rate with 0500 mg drug/kg body weight of animal
Naloxone: 21.00±0.99 → 25.75±0.54
Butorphanol: 27.17±0.50 → 32.29±0.92

Table I
Effects of opioid antagonists on *Uromastix heart and respiratory rates**

Drug	Dose mg/kg body weight	Heart Rate (M±SE)		Respiratory Rate (M±SE)	
		Before drug administration	After drug administration	Before drug administration	After drug administration
Naloxone	0.125	45.60±1.39	48.21±3.94	14.78 ±0.32	19.50 ±1.85
	0.250	62.17±1.40	70.83±0.40	19.17±0.17	34.67±2.65
	0.500	58.29±5.42	71.31±0.17	18.53±0.60	26.00±5.09
Pentazocine	0.125	51.31±0.36	59.50±1.57	10.08±0.50	12.72±0.56
	0.250	61.92±1.94	78.52±1.11	13.30±0.67	14.80±0.47
	0.500	67.23±2.09	80.27±1.47	16.27±0.73	18.67±0.65
Butorphanol	0.125	46.36±1.04	52.57±2.86	10.67±0.45	11.00±0.33
	0.250	61.90±0.10	74.00±0.52	18.58±1.16	22.45±0.58
	0.500	45.70±5.32	50.29±3.28	11.58±1.05	13.90±0.53

*Data were collected from 20 animals.

Table II
Effects of opioid antagonists on Rooste'rs* heart and respiratory rates

Drug	Dose mg/kg body weight	Heart Rate (M±SE)		Respiratory Rate (M±SE)	
		Before drug administration	After drug administration	Before drug administration	After drug administration
Naloxone	0.125	326.83±2.13	338.38±3.30	20.33±0.62	24.29±0.67
	0.250	310.82±2.48	312.67±3.46	21.03±1.24	23.73±0.54
	0.500	309.65±2.13	311.00±1.88	21.00±0.99	25.75±0.54
Pentazocine	0.125	308.71±2.02	318.33±4.81	23.30±0.40	27.23±0.88
	0.250	334.83±1.33	337.86±1.90	20.11±2.36	22.57±0.31
	0.500	347.40±1.66	350.73±2.04	21.78±0.55	22.93±0.45
Butorphanol	0.125	311.57±3.48	322.75±4.11	26.14±0.46	29.90±1.56
	0.250	323.1.82	335.50±5.06	26.25±0.50	33.82±1.18
	0.500	309.78±1.35	313.89±4.61	27.17±0.50	32.29±0.92

*Data were collected from 20 animals.

Discussion

The findings of these studies correlate with those of Willer and Albe-Fessard (1980). It is tempting to suggest that, in this study, three different mechanisms are involved in the bodily changes during stress-induced analgesia. The first one, corresponding to a reticular activation (Arousal System I) would induce mostly a significant modification in heart and respiratory rates along with inhibition of nociceptive reflexes. The second mechanism would involve a parallel and progressive release of endogenous opiates which would exert not only a moderating action on heart and respiration rates, but also a reinforcing effect on the inhibition of nociceptive reflexes. These hypotheses are confirmed by the effects induced by opioid antagonists which resulted in a double action: the first one (significant variations in the previous values of heart and respiration rates in most experiment Tables I and II) shows effectively that endogenous piates exerted a depressive modulation upon reticular structures responsible for the "Arousal System I". The second action revealed by the opioid antagonists concerned the facilitation of nociceptive reflexes as indicated by the animal's aggressive and emotional behaviour. The latter data suggest that endogenous opioids also exert a depressive effect on structures other than the

reticular formation, which would be responsible for hyperalgesia and behavioural changes. It is possible to suggest that these structures could correspond to the "Arousal System II". (Third mechanism described by Routtenberg, 1968), involving the limbic system since it is known that activation of these regions produces facilitation of nociceptive reflexes and aggressive behaviour (Willer and Albe-Fessard, 1980).

Results from a number of previous studies have suggested that endogenous opioid systems are involved in the mediation of aggressive behaviours (Rodgers and Randall, 1985) which might be the cause of stimulation of heart and respiratory rates in a few experiments of this study. Opioid antagonists have been shown to enhance, inhibit, have biphasic or to have no effects on shock-induced behaviour in various species and strains of laboratory animals (McGivern et al., 1981). It may be the cause of highly variable results between *Uronrastices* and *Gallus domesticus* obtained with different opioid antagonists used in this work. These highly variable results may, at least in part, relate to differences in dosage and species of animals.

Opioid antagonists altered the thermal response latencies, though this action could involve nonopiate-mediated effects of the antagonists (Sawynok et al., 1979). An insignificant degree of temperature rise was observed with all the three doses administered of each antagonist in both animals. Using the criterion of the antagonist's significant modification of the vegetative parameters (Tables I and II), the antinociception produced in these experiments appears to be mediated by endogenous opioid systems. This view gains strength from the finding that doses of naloxone as low as 0.1mg/kg body weight of animal can block the stress-induced analgesia (Terman et al., 1984; Jurna, 1988). Painful stimulation in the presence of an antagonist resulted in so intense pain that a nonopiate "backup" system was activated. It is especially interesting that the findings in this work reflect the operation of such a "backup" system which has the special feature of being relatively resistant to tolerance within limits. Opiate-mediated analgesia, in contrast, was subject to rapid tolerance (Greeley et al., 1988). The results of this work are further supported by the evidence showing that stress-induced analgesia may involve the activation of opioid and/or nonopioid mechanisms (Steinman et al., 1990). In this work, inescapable shocks were induced just above the tail on the dorsal lower trunk region of the animal, whereby the involvement of opioid mediated analgesia is confirmed.

The selective components of the stressor (i.e., electric shocks) used, i.e., the duration or controllability of the stressor, contributed to their antiopioid actions. The ability of a given stressor to activate both opioid and nonopioid forms of analgesia, combined with the observations that the nonopioid component of a stressor may antagonize opioid analgesia, suggests that there are functionally distinct roles for these multiple mechanisms (Steinman et al., 1990). The time course for the stress-induced analgesia was rapid; the latency for the response shortly tended to return to initial values. This suggests that the application of the stressor caused the release of endogenous opiates for only a

few minutes and that the peptides must, then, have been rapidly removed by degradative peptidases (Colett-Preverio et al., 1985). This rapid degradation of the opiate peptides was primarily responsible for the cessation of the analgesia coupled with a reduction in their release (Dalton and Widdowson, 1989). Repeated exposure to opioid-mediated stressor produced subsequent enhancement of opiate analgesia (Benedek and *Szikszay,* 1985). In conclusion, the results of these experiments show the involvement of both opiate — and nonopiate-mediated analgesia.

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