Pharmacology Biochemistry & Behavior, Vol. 22, pp. 249–254, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Differences of Alteration in Opioid Systems Induced by Conditioned Suppression and Electric Footshock in Mice

TSUTOMU KAMEYAMA, TOSHITAKA NABESHIMA AND KIYOFUMI YAMADA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences
Meijo University, Nagoya 468, Japan

Received 16 November 1983

KAMEYAMA, T., T. NABESHIMA AND K. YAMADA. *Differences of alteration in opioid systems induced by conditioned suppression and electric footshock in mice.* PHARMACOL BIOCHEM BEHAV 22(2) 249–254, 1985.—The motility, pain-threshold and opioid receptor activities of the synaptic membrane in mice showing conditioned suppression of motility were compared with those in mice given only electric footshock. Electric footshock caused analgesia and a decrease in motility, both of which were partially reversed by administration of high doses of naloxone. In contrast, mice exhibited a marked suppression of motility (conditioned suppression) but not analgesia when placed in the same environment 24 hr after the electric footshock in which the animals received the electric footshock. In the electric footshock group, the [^{3}H]-naloxone binding capacity at low affinity site was increased. These results suggest that the increase in [^{3}H]-naloxone binding capacity may play an important role in the behavioral changes of electric footshock group, but not conditioned suppression group.

Conditioned suppression Electric footshock Motility Opioid activity Pain-threshold

RATS and mice subjected to electric footshock exhibit a marked suppression of motility when placed in the same environment in which they received the electric footshock. This suppression is a stable response and a conditioned emotional response to the environment (conditioned suppression) [1, 15, 16, 17, 18, 25, 26]. Furthermore, it has been reported that this conditioned suppression is attenuated by the pretreatment of morphine and cyclazocine [16,25] and that the effect of morphine is antagonized by naloxone [16]. Thus, endorphinergic neuronal activity may be altered in mice showing a conditioned suppression of motility. On the other hand, accumulated evidence indicates that electric footshock produces analgesia and a decreased motility, and these responses induced by electric footshock may be mediated by the endogenous opioid systems since they were totally or partially antagonized by naloxone [6, 11, 24]. Furthermore, the decreases in exogenous opiate binding, presumably due to an increased release of endogenous opioids, occur in cases of stress-induced analgesia [7, 10, 22]. We attempted to determine whether endogenous opioid neuronal activity (as evidenced by [^{3}H]-naloxone's binding capacity to synaptic membrane of mice showing conditioned suppression of motility) is altered, and to compare the motility, pain-threshold and opioid binding activity to synaptic membrane of mice showing conditioned suppression with those of mice given electrical footshock.

METHOD

Subjects

Male ddY mice, 8 weeks of age, were housed in groups of 10 in a controlled environment (22±1°C, 50±5%) and provided food and water ad lib. The room lights were off between 8:00 p.m. and 8:00 a.m.

Apparatus

Experiments were carried out using a transparent acrylic rectangular cage (23×28×12 cm) with a metal wire floor. The apparatus was located in a sound-proof room lit with a 20 W bulb. Electric shock (0.1 Hz, 200 msec, 300 VDC) was delivered through an isolated stimulater (Nihon Koden, Tokyo, Japan). The resistance, when an animal was placed in a test cage, varied between 100 and 250 kΩ. Therefore, an animal received electric shocks in the range of 1.2–3 mA.

Test Procedure

Behavioral testing was done between 9:00 a.m. and 4:00 p.m. A mouse was left in the experimental apparatus for 6 min, given electric footshock and then placed in one of three groups of 10 mice. Each mouse of electric shock groups was tested for pain-threshold and motility immediately (ES_0 group) or 24 hr (ES_{24} group) after the shock treatment. Each mouse in a third group was again placed in the same apparatus 24 hr after the shock treatment, but this time no electric footshock was given (conditioned suppression (CS) group). Corresponding control groups not given electric shock were also run.

Measurement of Motility and Pain-Threshold

Pain-threshold was measured by the method of D'Amour

TABLE 1

COMPARISON OF MOTILITY AND PAIN-THRESHOLD OF MICE BETWEEN ELECTRIC FOOTSHOCK AND CONDITIONED SUPPRESSION GROUPS

	Motility meter	Motility median (range)	N	% of control	Pain-threshold (sec) median (range)	N
Control	Automex	203 (70–257)	11	100.0	3.0 (1.7–4.6)	8
ES_0		28.5 (0–73)*	10	14.0	5.2 (1.8–15.0)‡	27
ES_{24}		151 (40–235)	11	74.4	3.0 (2.2–6.6)	12
Control	Opto-Varimex	2789 (1662–3730)	11	100.0	—	
CS		528 (6–1196)†	9	18.9	3.6 (2.4–4.0)	8

Motility of mice in conditioned suppression (CS) and electric footshock (ES_0 and ES_{24}) groups was recorded as described in the Method section by an Opto-Varimex and Automex for 6 min, respectively. Pain-threshold of mice was measured by the method of D'Amour and Smith. Values are the median and range. N: number of animals. *$p<0.01$ compared to the control (Automex) group. †$p<0.01$ compared to the control (Opto-Varimex) group. ‡$p<0.01$ compared to the control group.

and Smith [9]. Tail-flick latency in the electric footshock groups was measured immediately or 24 hr after the shock treatment. Motility of the electric footshock groups was measured in a different cage from the shock treatment apparatus using an Automex (Columbus Instruments, OH) for 6 min immediately after the measurement of pain-threshold. The motility of the conditioned suppression group was measured for 6 min in the shock treatment apparatus surrounded by an Opto-Varimex (Columbus Instruments, OH), a locomotor activity meter. Immediately after the measurement of motility, the pain-threshold was measured.

Drugs

Naloxone HCl (Endo Laboratories, Inc., Garden City, NY) was dissolved in saline and administered intraperitoneally at the indicated doses 15 min prior to measurement of the pain-threshold and motility. The doses refer to the indicated salt forms. [^{3}H]-naloxone (specific activity: 70 Ci/mmol) was obtained from New England Nuclear. Its radiochemical purity was verified by thin-layer chromatography in an appropriate solvent system.

Preparation of Synaptic Membrane

In the electric footshock groups, the mice were sacrificed by decapitation immediately or 24 hr after the shock treatment. In the conditioned suppression group, the mice were sacrificed 6 min after being returned to the shock treatment apparatus. The mice of the corresponding control groups were also sacrificed and the whole brains were rapidly taken out. The whole brains, excluding the cerebellum, were rapidly removed and used for synaptic membrane preparation following the methods of Gray and Whittaker [13] and Terenius [30]. Briefly, about 4.5 g of pooled brains was homogenized and centrifuged at 1,000 g for 10 min. The supernatant fraction was collected and centrifuged at 20,000 g for 20 min to obtain a pellet containing the crude mitochondrial fraction (P_2 fraction). The P_2 fraction was subjected to osmotic shock by adding 10 ml of deionized-distilled water. The suspension was centrifuged at 12,000 g for 20 min. The carefully decanted supernatant, suitably divided, was layered over a discontinuous gradient consisting of 0.6 M and 1 M sucrose in 5 mM Tris-HCl buffer (pH 7.4), and centrifuged in a Beckman SW-27 rotor at 100,000 g for 60 min (Beckman L8-80 ultracentrifuge). The band between 0.6 and 1 M sucrose (P_2B fraction) was collected. The pooled P_2B fraction was divided into suitable aliquots and stored at −60°C. Storing of the membrane in this fashion for 8 weeks did not significantly alter the binding activity. Before the binding assay, the aliquots were diluted with 10 volumes of 25 mM Tris buffer (pH 7.4), incubated at 37°C for 30 min to remove endogenous opioids and then centrifuged at 25,000 g for 20 min, giving a pellet [29]. This pellet was re-homogenized with a suitable volume of the Tris buffer to obtain the final membrane preparation for assay; this contained 0.15–0.25 mg/ml protein.

Binding Assay

Opioid receptor binding was initiated by the addition of 0.2 ml of the membrane preparation to a mixture containing the required final concentration of [^{3}H]-naloxone in a total volume of 1.0 ml. Incubation was carried out at 25°C for 30 min. The binding reaction was stopped rapidly by filtering through a Whatman GF/B filter. The filter was washed twice with 5 ml of ice-cold 25 mM Tris buffer (pH 7.4). The conditions adopted here for the binding assay were derived from preliminary studies and were found to be optimum [29]. The filters were transferred to scintillation counting vials containing 10 ml of ACS II (Amersham Co., Arlington Heights, IL). The vials were shaken for 60 min and the radioactivity was measured using a Model 3255 Tri-Carb Liquid Scintillation Spectrometer System at a counting efficiency of 31%. Specific binding was defined as the difference in amount of [^{3}H]-naloxone bound to the membrane in the presence or absence of 5 μM non-radioactive naloxone. In experiments, 8 concentrations of [^{3}H]-naloxone ranging from 0.2 to 20 nM were used. The protein concentration was determined by the method of Lowry *et al.* [21].

Statistics

The analysis of biphasic Scatchard plots was performed by the method of Rosenthal [28] using computer program to obtain the dissociation constant (Kd) and the maximum binding capacity (Bmax). When applicable, statistical significances were analyzed by a one-way-analysis of variance followed by a Dunnett's t for biochemical data or Mann-

TABLE 2

EFFECT OF NALOXONE ON MOTILITY AND PAIN-THRESHOLD OF MICE IMMEDIATELY AFTER SHOCK TREATMENT (ES_0)

Behavior	Dose (mg/kg, IP)	Control median (range)	N	% of control	ES_0 median (range)	N	% of control
Motility							
Naloxone	0	162 (58–325)	27	100.0	46 (0–139)†	34	28.4
	1	148 (62–363)	10	91.4	62 (3–118)†	10	38.3
	10	150 (107–241)	8	92.6	67 (21–166)†‡	16	41.4
	30	196 (51–253)	10	121.0	86 (50–128)*‡	10	53.1
Pain-threshold (sec)							
Naloxone	0	2.7 (1.5–4.6)	27		5.2 (2.0–15.0)†	34	
	1	3.0 (2.1–4.3)	10		12.0 (4.5–15.0)†	10	
	10	2.9 (2.1–3.5)	8		4.2 (3.5– 5.0)†§	16	
	30	2.5 (1.7–3.4)	10		3.7 (3.0– 7.8)†‡	10	

Naloxone was administered IP 15 min prior to the measurement of pain-threshold and motility. Motility and pain-threshold were measured as described in the Method section. Values are the median and range. N: number of animals. *$p<0.05$, †$p<0.01$ compared to control group of naloxone 0 mg/kg. ‡$p<0.05$, §$p<0.01$ compared to electric shock group of naloxone 0 mg/kg.

TABLE 3

EFFECT OF NALOXONE ON MOTILITY OF MICE IN CONDITIONED SUPPRESSION (CS) GROUP

Drug	Dose (mg/kg, IP)	Control median (range)	N	% of control	CS median (range)	N	% of control
Naloxone	0	2789 (1662–3730)	11	100.0	528 (6–1196)*	9	18.9
	1	2689 (1150–4026)	9	96.7	295 (26–1246)*	8	10.6
	10	2453 (1046–3209)	8	88.0	356 (38– 747)*	10	12.8
	30	1977 (679–3773)	9	70.9	183 (24–2108)*	10	6.6

Naloxone was administered IP 15 min prior to the measurement of motility. Values are the median and range. N: number of animals. *$p<0.01$ compared to control group of naloxone 0 mg/kg.

Whitney's U-test for behavioral data. A p value of 0.05 between two means was considered significant.

RESULTS

Comparison of Motility and Pain-Threshold Between Electric Footshock and Conditioned Suppression Groups

The mice given electric footshock showed a significant decrease in motility (14.0% of the control) immediately after the shock treatment (ES_0). The decrease of motility returned to the control level 24 hr after the shock treatment (ES_{24}). However, when the mice were returned to the same apparatus in which they had been given electric shock, they exhibited a marked suppression of motility (18.9% of the control) even 24 hr after the shock treatment (conditioned suppression group, CS) (Table 1). In the pain-threshold test, mice given electric footshock showed a significant increase in tail-flick latency (5.2 (1.8–15.0) sec) immediately after the shock treatment compared with the control value (3.0 (1.7–4.6) sec). However, in the other groups the tail-flick latencies were not significantly different from that of the control animals (Table 1).

Effects of Naloxone on Motility and Pain-Threshold in Electric Footshock and Conditioned Suppression Groups

The decrease in motility and the increase in tail-flick latency of mice immediately after the shock treatment were observed again and partially antagonized by high doses of naloxone (10 and 30 mg/kg) but not by a low dose (1 mg/kg) (Table 2). On the contrary, the marked suppression of motility in the conditioned suppression group was resistant even to 30 mg/kg of naloxone (Table 3).

Opioid Binding Activities in Electric Footshock and Conditioned Suppression Groups

To determine the possible functional changes in endogenous opioid systems, the opioid binding activities of the synaptic membranes from the electric footshock and conditioned suppression groups were compared with those of the control groups. As shown in Table 4, the amounts of binding of [^{3}H]-naloxone significantly increased in ES_0 group at the concentrations of 1 and 10 nM naloxone compared with control groups. The increase of naloxone binding at 1 nM returned into the control level 24 hr after the electric

TABLE 4

[^{3}H]-NALOXONE BINDING ACTIVITY OF BRAIN SYNAPTIC MEMBRANE IN CONTROL, ES_0, ES_{24} AND CS GROUPS

Treatment	N	Concentration of [^{3}H]-naloxone (nM)	Specific Binding (f mole/mg protein)
Control	3	High (1)	47.7 ± 1.16
	3	Low (10)	347.0 ± 8.47
ES_0	3	High (1)	58.1 ± 1.68*
	3	Low (10)	427.7 ± 9.62*
ES_{24}	3	High (1)	43.2 ± 1.52‡
	3	Low (10)	266.8 ± 14.2*‡
CS	3	High (1)	56.6 ± 1.38*§
	3	Low (10)	376.7 ± 12.8†§

Fixed concentration of [^{3}H]-naloxone was assayed with the synaptic membrane from control, ES_0, ES_{24} and CS groups as described in Method section. Values are mean ± S.E. N: the number of independent experiments, each in triplicate. *$p<0.01$ compared to the control group. †$p<0.05$, ‡$p<0.01$ compared to the ES_0 group. §$p<0.01$ compared to the ES_{24} group.

TABLE 5

THE DISSOCIATION CONSTANTS (Kd) AND THE MAXIMUM BINDING CAPACITY (Bmax) OF BRAIN SYNAPTIC MEMBRANE IN CONTROL, ES_0, ES_{24} AND CS GROUPS

Treatment	N	Affinity	Kd (nM)	Bmax (f mole/kg protein)
Control	3	High	0.28 ± 0.06	16.2 ± 3.3
		Low	21.1 ± 1.0	818.0 ± 85.9
ES_0	3	High	0.24 ± 0.05	23.1 ± 4.8
		Low	20.3 ± 0.9	1119.8 ± 57.0*
ES_{24}	3	High	0.28 ± 0.04	18.8 ± 2.0
		Low	18.5 ± 5.0	685.0 ± 102.4†
CS	3	High	0.25 ± 0.02	22.2 ± 5.5
		Low	20.5 ± 2.9	951.7 ± 239.2

The Kd and Bmax were determined by the method of Rosenthal (1967) using a computer program. Values are mean ± S.E. N: the number of independent experiments, each in triplicate. *$p<0.05$ compared to the control group. †$p<0.05$ compared to the ES_0 group.

shock (ES_{24}). [^{3}H]-naloxone binding in CS group also significantly increased compared with ES_{24} group at both concentrations of naloxone. To clarify whether affinity and/or binding capacity were modified by the electric shock, saturation curves for [^{3}H]-naloxone were generated using several concentrations (0.2–20 nM). The Scatchard plots were curvilinear, which suggested the presence of at least two classes of binding sites, namely high- and low-affinity sites. The Kd and Bmax values for high- and low-affinity sites in each group are presented in Table 5. The maximum binding capacity for low-affinity site of the synaptic membranes in creased signficiantly without altering the affinity immediately after the shock treatment. Similarly, a tendency of increase in the number of binding site was also observed in the conditioned suppression group, but not significant.

DISCUSSION

It has been reported that animals subjected to stressful events show a wide range of phenomena such as changes in body temperature [2], analgesia [3,24] and catalepsy [19], and that these phenomena are mediated by the endogenous opioid systems, based on the evidence of the antagonistic effect of naloxone and cross-tolerance to morphine. In addition, chronic ethylketocyclazocine or pentazocine administration have attenuated electric shock-induced immobilization [24]. In the present experiments, mice, subjected to electric footshock, showed a significant increase in the pain-threshold and a decrease in the motility immediately after shock treatment, both of which were partially antagonized by a high dose of naloxone. These results are in agreement with the findings of other investigators and us [12, 24, 31]. In addition, in mice showing a pronounced analgesic effect and decreased motility immediately after the shock treatment, [^{3}H]-naloxone binding was significantly increased compared with the control group, and the increased Bmax was returned to the control level 24 hr after the shock treatment. At this time, the analgesic effect and decreased motility were no longer observed. Thus, it appears that the analgesia and decreased motility induced by electric footshock may be mediated in part by the endogenous opioid systems. In addition, since it has been reported that acute treatment with morphine induces an increase in the number of binding sites [27,29], the increased binding capacity induced by electric footshock may relate to the increase in endogenous opioid activity. Madden *et al.* [22] reported a decrease in maximum binding due to an increase in receptor occupation by endogenous opioids, presumably caused by increased endogenous opioid release in rats with footshock-induced analgesia. They also reported that preincubation of whole-brain homogenates at 37°C equalized the [^{3}H]-naloxone binding capacity in the stress and control groups. As described in the Method section, the synaptic membrane preparation used in this experiment was preincubated at 37°C before the binding assay to eliminate endogenous opioids. Therefore, this discrepancy between Madden *et al.*'s and our results may be due to the difference in animal species (i.e., rats and mice) or to the different conditions under which electrical shock was given.

A marked suppression of motility but not analgesia was observed when mice were placed again in the same apparatus in which they had received electric footshock. In contrast with the decrease in motility of the electric shock group, this response in the conditioned suppression group was resistant to the opiate antagonist, naloxone. In addition, there was no difference in [^{3}H]-naloxone specific binding to synaptic membranes between the conditioned suppression and control groups. However, this conditioned suppression is attenuated by the pretreatment of morphine and cyclazocine as described in introduction [16,25]. Therefore, the wide spectrum of responses induced by stress may be due to the considerable heterogeneity of endogenous opioids. Three types of specific receptors in nervous tissue are suggested by Martin *et al.* [23] to mediate the agonist activity of opioid derivatives. These receptors are named for prototypic drugs which produced distinct patterns of physiological effects in spinal dogs: mu for morphine, kappa for ketocyclazocine and sigma for SKF-10047. Within this framework, the mu receptors are involved in the production of supraspinal analgesia, respiratory depression, euphoria and physical dependence. Some

drugs may also relate to the kappa receptors, which when activated, induce spinal analgesia, miosis and sedation. Activation of sigma receptors causes dysphoria and hallucinations, as well as respiratory and vasomotor stimulatory effects. In addition, the discovery of the enkephalins [14] has led to the fourth classification for peptides: delta [20]. The behavioral [8,32] and biochemical [4, 5, 33] actions of agonists related to delta, kappa and sigma receptors in rodents are reversed hardly by naloxone. Therefore, the doses of naloxone required to block the footshock-induced effects suggest a contribution of delta, kappa and sigma receptors instead of mu receptor. On the other hand, the magnitude of analgesia, but not motor suppression of ES_0 group, is significantly less in morphine-tolerant mice than non-tolerant mice. On the contrary, the magnitude of motor suppression, but not analgesia, is significantly less in ethylketocyclazocine- and pentazocine-tolerant mice than non-tolerant mice [24]. Furthermore, the potency of cyclazocine, kappa and/or sigma agonist, to reduce the conditioned suppression in mice is more than that of morphine [25,34]. Therefore, it is possible that the analgesic effect of ES_0 group may be mediated in part at the mu and/or delta receptors, while the suppression of motility in the ES_0 and CS groups may be mediated at the kappa and/or sigma receptors. To clarify this point, the binding assay of [^{3}H]-ethylketocyclazocine and [^{3}H]-SKF-10047 to membrane prepared from CS group should be done.

In conclusion, an increase in analgesic threshold and a decrease in motility were observed in ES_0 group, and both could be partially reversed by naloxone. On the contrary, mice in CS group, exhibited a marked suppression of motility but not analgesia, and this suppression of motility was resistant to naloxone. Further, in the synpatic membranes of ES_0 group, there was an increase in the maximum binding site although the affinity was not altered. The exact role of the endogenous opioid system remains obscure, but these results suggest that an involvement of mu and/or delta receptors may be important in behavioral changes induced by electric footshock, but not conditioned suppression.

ACKNOWLEDGEMENTS

We thank Mr. K. Matsuno and Mr. M. Suzuki for preparing the computer program of Rosenthal plots and Mr. H. Kamei for technical assistance.

REFERENCES

1. Babbini, M., M. Gaiardi and M. Bartoletti. Effects of morphine on a quickly learned conditioned suppression in rats. *Psychopharmacologia* **33**: 329–332, 1973.
2. Bläsig, J., V. Höllt, U. Baverle and A. Herz. Involvement of endorphin in emotional hyperthermia of rats. *Life Sci* **23**: 2525–2532, 1978.
3. Buckett, W. R. Peripheral stimulation in mice induces short-duration analgesia preventable by naloxone. *Eur J Pharmacol* **58**: 169–178, 1979.
4. Chang, K. J., B. R. Cooper, E. Hazum and P. Cuatrecasas. Multiple opiate receptors: different regional distribution in brain and differential binding of opiates and opioid peptides. *Mol Pharmacol* **16**: 91–104, 1979.
5. Chang, K. J. and P. Cuatrecasas. Multiple opiate receptors: enkephalin and morphine bind to receptors of different specificity. *J Biol Chem* **254**: 2610–2618, 1979.
6. Chester, G. B. and B. Chan. Footshock induced analgesia in mice: its reversal by naloxone and cross-tolerance with morphine. *Life Sci* **21**: 1569–1574, 1977.
7. Christie, M. J., G. B. Chesher and K. D. Bird. The correlation between swim-stress induced antinociception and [^{3}H]leu-enkephalin binding to brain homogenates in mice. *Pharmacol Biochem Behav* **15**: 853–857, 1981.
8. Collier, H. O. J. and C. Schneider. Some agonist and antagonist properties of morphine agonists in rodents. In: *Agonist and Antagonist Action of Narcotic Analgesic Drugs*, edited by H. W. Kosterlitz, H. O. J. Collier and J. E. Villarreal. Baltimore: University Park Press, 1972, pp. 128–135.
9. D'Amour, F. E. and E. L. Smith. A method for determining loss of pain sensation. *J Pharamcol Exp Ther* **72**: 74–79, 1941.
10. DeVries, G. N., W. T. Chance, W. R. Payne and L. A. Rosencrans. Effect of autoanalgesia on CNS enkephalin receptors. *Pharmacol Biochem Behav* **11**: 741–744, 1979.
11. Fanselow, M. S. and R. C. Bolles. Naloxone and shock-elicited freezing in rat. *J Comp Physiol Psychol* **39**: 736–744, 1979.
12. Frenk, H., J. Engel, Jr., R. Ackermann, Y. Shavit and J. C. Liebeskind. Endogenous opioids may mediate post-ictal behavioral depression in amygdaloid-kindled rats. *Brain Res* **167**: 435–440, 1979.
13. Gray, E. G. and V. P. Whittaker. The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. *J Anat* **96**: 79–88, 1962.
14. Hughes, J., T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan and H. R. Morris. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258**: 577–579, 1975.
15. Kameyama, T. and M. Nagasaka. Effects of apomorphine and diazepam on a quickly learned conditioned suppression in rats. *Pharmacol Biochem Behav* **17**: 59–63, 1982.
16. Kameyama, T. and M. Nagasaka. The effects of analgesics on quickly learned conditioned suppression in mice. *Neuropharmacology* **21**: 1283–1289, 1982.
17. Kameyama, T. and M. Nagasaka. Effects of apomorphine and methamphetamine on a quickly-learned conditioned-suppression response in rats. *Neuropharmacology* **22**: 813–817, 1983.
18. Kameyama, T. and M. Nagasaka. Effect of naloxone on a quickly learned conditioned suppression in rats following cessation of chronic haloperidol treatment. *Brain Res* **275**: 389–391, 1983.
19. Katz, R. J. Stress induced facilitation of opiate catalepsy in rat. *Prog Neuropsychopharmacol* **4**: 309–312, 1980.
20. Lord, J., D. Waterfield, J. Hughes and H. W. Kosterlitz. Endogenous opioid peptides: multiple agonists and receptors. *Nature* **267**: 495–499, 1977.
21. Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall. Protein measurement with the folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
22. Madden, J., H. Akil, R. L. Patrick and J. Barchas. Stress induced parallel changes in central opioid levels and pain responsiveness in rat. *Nature* **265**: 358–360, 1977.
23. Martin, W. R., C. G. Eades, L. A. Thompson, R. E. Huppler and P. E. Gilbert. The effects of morphine- and nalorphine-like drugs in the non-dependent and morphine dependent chronic spinal dog. *J Pharmacol Exp Ther* **197**: 517–522, 1976.
24. Nabeshima, T., K. Yamada and T. Kameyama. Contribution of different opioid systems to footshock-induced analgesia and motor suppression. *Eur J Pharmacol* **92**: 199–205, 1983.
25. Nabeshima, T., K. Yamada and T. Kameyama. Effects of opiate agonists on the conditioned suppression in motility of mice. *Neurosci Lett* **39**: 301–306, 1983.
26. Nagasaka, M. and T. Kameyama. Effects of diazepam, meprobamate, chlorpromazine and apomorphine on a quickly learned conditioned suppression in mice. *J Pharmacobio Dyn* **6**: 523–526, 1983.

27. Pert, C. B., G. W. Pasternak and S. H. Snyder. Opiate agonists and antagonists discriminated by receptor binding in brain. *Science* **182:** 1359–1361, 1973.
28. Rosenthal, H. E. A graphic method for the determination and presentation of binding parameters in a complex system. *Anal Biochem* **20:** 525–532, 1967.
29. Sivam, S. P., T. Nabeshima and I. K. Ho. Alterations of synaptic high- and low-affinity opiate binding sites after acute and chronic morphine administration in mice. *Prog Neuropsychopharmacol* **6:** 119–127, 1982.
30. Terenius, L. Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. *Acta Pharmacol Toxicol (Copenh)* **32:** 317–320, 1973.
31. Urca, G., J. Yitzhaky and H. Frenk. Different opioid systems may participate in post-electroconvulsive shock (ECS) analgesia and catalepsy. *Brain Res* **219:** 385–396, 1981.
32. Ward, S. J., G. Metcalf and J. M. H. Rees. Effects of μ, κ, and σ agonists in rodents. *Dev Neurosci* **4:** 497–498, 1978.
33. Wood, P. L., M. Stotland, J. W. Richard and A. Rackham. Actions of mu, kappa, sigma, delta and agonist/antagonist opiates on striatal dopaminergic function. *J Pharmacol Exp Ther* **215:** 697–703, 1980.
34. Yamada, K., T. Nabeshima and T. Kameyama. Effects of opiate agonists and antagonists on the conditioned suppression in motility of mice. *Neurosciences (Kobe)* **9:** 53–54, 1983.