Naunyn-Schmiedeberg's Arch Pharmacol (1986) 332:376–379

Naunyn-Schmiedeberg's
Archives of
Pharmacology

An analgesic effect of enkephalinase inhibition is modulated by monoamine oxidase-B and REM sleep deprivations

Otas Eghe Ukponmwan, Joze Rupreht, and Mihailo Dzoljic

Department of Pharmacology, Faculty of Medicine, Erasmus University Rotterdam, P.O. Box 1738, NL-3000 DR Rotterdam, The Netherlands

Summary. Both the MAO-B inhibitor deprenyl (2.5–10 mg/kg, ip, 60 min prior) and the MAO-B substrate β-phenylethylamine (PEA, 40 μg, icv) potentiated the analgesic action of the enkephalinase inhibitor phosphoramidon (250 μg, icv) in animals allowed normal sleep. The enhancing effect of PEA on phosphoramidon analgesia was further potentiated by deprenyl (5 mg/kg, ip) pretreatment. Deprenyl (5 mg/kg, ip) or PEA (40 μg, iv) given alone did not induce analgesia in animals allowed undisturbed sleep.

REM sleep deprivation (REMSD) decreased the basal pain threshold and abolished the analgesic effect of phosphoramidon. The administration of deprenyl and/or PEA failed to restore the analgesic effect of phosphoramidon in REM sleep deprived animals.

The results indicate that excess PEA has a stimulatory effect on the analgesic activity of endogenously released enkephalins in rats allowed undisturbed sleep but not in REM sleep deprived animals.

It is suggested that the failure of phosphoramidon to induce analgesia after REMSD, is probably due to a functional insufficiency of an enkephalinergic system.

Key words: Sleep – Enkephalinase inhibition – MAO-B – Nociception

Introduction

Two forms of monoamine oxidase (MAO) are present in the mammalian brain, MAO-A and MAO-B. Serotonin, dopamine and noradrenaline are preferred substrates for MAO-A, while MAO-B shows selectivity for β-phenylethylamine (PEA) (Yang and Neff 1974; Garrick and Murphy 1980). Several lines of evidence suggested that inhibition of MAO activity increased the pharmacological effects/toxicity of opiates in patients (Taylor 1962) and animals (Iwamoto and Ho 1972; Boden et al. 1984), although this effect was only seen when both MAO-A and MAO-B were inhibited (Jounela et al. 1977). Nevertheless some interactions between MAO-B inhibitors and opiates/endorphins have been reported. For example, an inhibition of MAO-B or excess of PEA (the substrate for MAO-B) potentiated the analgesia induced by exogenously administered opiates/opioids (Fuentes et al. 1977; Garzon et al. 1980). In addition, some pharmacological actions of PEA can be modulated by opioid receptor blockade (Kubota et al. 1982; Dourish and Cooper 1984) suggesting a possible interaction between PEA and opioid receptors.

This study was undertaken to clarify the relationship between MAO-B and the analgesic effect of endogenous (synaptic) enkephalins following administration of an enkephalinase inhibitor to rats allowed undisturbed sleep and animals subjected to REMSD. In these experiments REM sleep deprived rats were used because it has been shown that both MAO-B inhibitors and REMSD possess antidepressant activity (Mann and Gershon 1980; Vogel et al. 1980) and modulate the analgesic action of opiates/opioids (Garzon et al. 1980: Ukponmwan et al. 1984a).

Send offprint requests to M. Dzoljic at the above address

Materials and methods

Adult, male Wistar rats weighing 150–175 g were used in this study. Drugs were injected intracerebroventricularly (icv), when required, via a stainless steel cannula implanted in the lateral ventricle. Correct placement of icv cannula was verified using the procedure recently described (Ukponmwan et al. 1985). A 4 day recovery period was allowed after cannula implantation before the experiments were commenced.

REM sleep deprivation. REMSD (96 h) and the corresponding stress-control, were carried out as previously described (Ukponmwan et al. 1984a) using a modification of the method of Mendelson (1974). This method is known to selectively deprive rats of REM sleep after 96 h (Mendelson et al. 1974). Throughout this study, all animals were maintained in a constant environment room with ambient temperature 22 ± 1°C and automatically regulated light-dark cycle of 12 h (light period 9.00–21.00 h). Food and clean drinking water were available ad libitum.

Assessment of nociception. Pain sensitivity to noxious paw pressure was assessed between 13.00–16.00 h using the analgesiometric technique of Randall and Selitto (1957). Nociception was measured 15, 30, 60 and 120 min after drug administration and expressed as analgesiometric scores (AMS) g mm^{-2} pressure. The cut off value was measured by a squeak or paw-withdrawal. Animals scoring above 150 g mm^{-2} during control testing were not used for further experimentation.

Drugs. The following drugs were used in this study: phosphoramidon (Peninsula Laboratories, San Carlos, CA, USA), deprenyl (Chinoin, Budapest, Hungary) and β-phenylethylamine (PEA, Sigma, St. Louis, MO, USA).

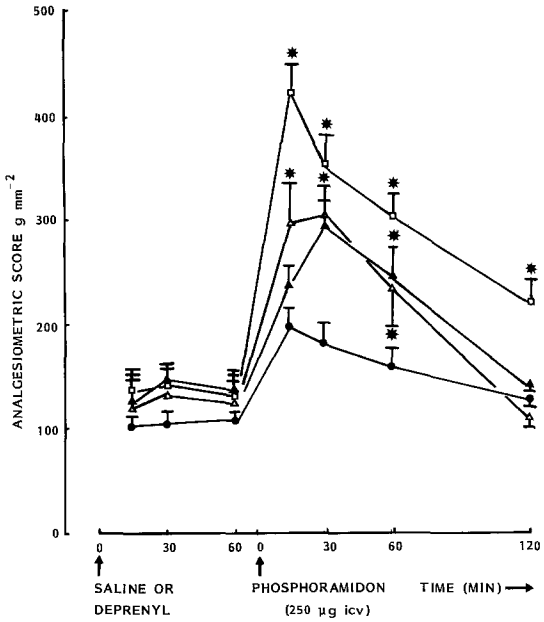

Fig. 1. The effect of deprenyl on the analgesic action of the enkephalinase inhibitor phosphoramidon in rats allowed undisturbed sleep. Nociception was determined by withdrawal of hind paw from pressure stimulation (modified Randall-Selitto test). Each point is mean ± SEM at each time point. Note that deprenyl (2.5–10 mg/kg, ip, 60 min prior) potentiated the analgesic effect of phosphoramidon (250 µg icv) in a dose-related manner. (*) Indicate significant difference from saline pretreated group ($p < 0.05$). The number of animals per treatment group is indicated in parenthesis. [●——●, saline 1.0 mg/kg ip (13); ▲——▲, deprenyl 2.5 mg/kg ip (9); △——△, deprenyl 5.0 mg/kg ip (7); □——□, deprenyl 10.0 mg/kg ip (7)]

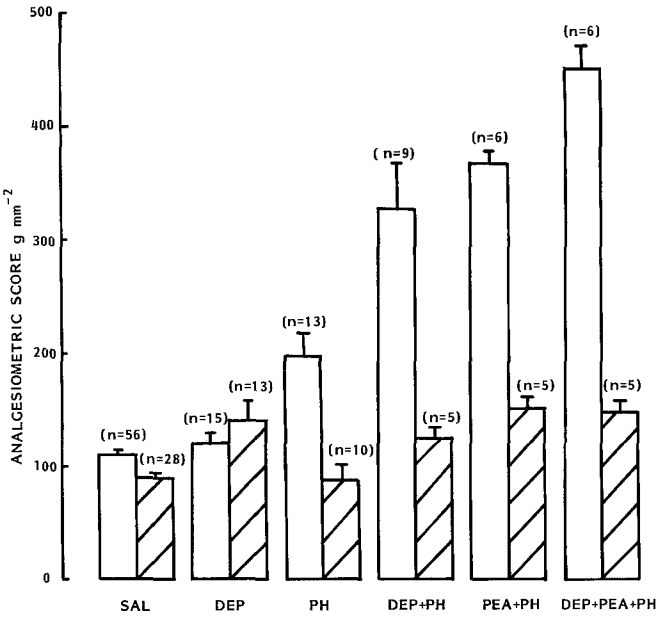

Fig. 2. The effects of deprenyl (*DEP*), REM sleep deprivation (*REMSD*) and β-phenylethylamine (*PEA*) on the analgesic effect of the enkephalinase inhibitor phosphoramidon (*PH*). Nociception was determined by withdrawal of hind paw from pressure stimulation (modified Randall-Selitto test). The analgesic score was measured 60 min after DEP and 15 min after PH or saline (2 µl icv, *SAL*). Each bar is the mean analgesic score ± SEM. The number of rats per group is indicated in parenthesis. Note the following: a) DEP (5 mg/kg, ip) and/or PEA (40 µg, icv) significantly potentiated the analgesic effect of PH in animals allowed undisturbed sleep; b) REMSD decreased the basal pain threshold compared to rats allowed undisturbed sleep; c) DEP and/or PEA did not alter the blockade of PH-induced analgesia by REMSD. The levels of significance are given in the trext [□, rats allowed undisturbed sleep; ▨, REM sleep deprived animals; *DEP*, deprenyl (5 mg/kg ip); *PH*, phosphoramidon (250 µg icv); *PEA*, β-phenylethylamine (40 µg icv)]

Drugs for icv or ip administration were dissolved in physiological saline and administered in volumes of 2 µl or 500 µl respectively. Since the half-life of PEA in the brain is known to be very short (Wu and Boulton 1975) this substance was given 5 min after phosphoramidon and the pain threshold was measured 10 min later.

Statistics. The significance of differences between the analgesic scores obtained after different treatments was evaluated by Duncan's new multiple range test, once a one way analysis of variance (ANOVA) had revealed that samples represented different populations (Steel and Torrie 1980; Saxena 1985). Statistical significance was accepted at *P*-values of 0.05 or less (two tailed).

Results

A significant difference in the analgesic scores across the various groups and time intervals was found after the administration of phosphoramidon (Fig. 1). Similarly the effect of pretreatment with deprenyl and/or β-phenylethylamine (PEA) on phosphoramidon induced analgesia was significant across the treatment groups (Fig. 2, $p < 0.01$).

The effects of deprenyl and/or β-phenylethylamine on phosphoramidon- induced analgesia in animals allowed normal sleep

The administration of the enkephalinase inhibitor phosphoramidon (Hudgin et al. 1981) (250 µg, icv) significantly increased the pain threshold to paw pressure. The most prominent analgesic effect was registered between 15–30 min after drug administration (Fig. 1).

Deprenyl the MAO-B inhibitor (2.5–10 mg/kg, ip 60 min prior) potentiated the analgesic effect of phosphoramidon in a dose related manner (Fig. 1). Similarly the MAO-B substrate PEA (40 µg, icv, 5 min post-phosphoramidon) also enhanced the phosphoramidon-induced analgesia (Fig. 2, $p < 0.05$). Pretreatment with deprenyl (5 mg/kg, ip, 60 min prior) further increased the potentiating effect of PEA (40 µg, icv) on the analgesic action of phosphoramidon (Fig. 2, $p < 0.05$). Neither deprenyl (5 mg/kg, ip) (Fig. 2, $p < 0.05$) nor PEA (40 µg, icv, data not shown) induced analgesia in animals allowed undisturbed sleep.

The effects of deprenyl and β-phenylethylamine on phosphoramidon-induced analgesia in REM sleep deprived animals

The basal nociceptive threshold in REM sleep deprived rats was slightly, but significantly, lower than in animals allowed undisturbed sleep (Fig. 2, $p < 0.05$). Deprenyl (5 mg/kg, ip, 60 min prior) induced a slight increase in the pain threshold of REMSD animals. A similar effect was observed during the first 10 min after PEA (40 µg, icv) administration (data

not shown). However, the analgesic scores of REM sleep deprived rats treated with deprenyl (5 mg/kg, ip, Fig. 2) or PEA (40 μg, icv, not shown) were not different from those of control animals (rats allowed undisturbed sleep). Phosphoramidon (250 μg, icv) had no analgesic action in animals subjected to REMSD (Fig. 2). The analgesic score of REM sleep deprived rats after administration of deprenyl (5 mg/kg, ip, 60 min prior) and/or PEA (40 μg, icv, 5 min post-phosphoramidon) plus phosphoramidon (250 μg, icv), was not different from those treated with deprenyl alone (Fig. 2, $p > 0.05$).

Discussion

In the animals allowed undisturbed sleep, the antinociceptive effect of the enkephalinase inhibitor phosphoramidon was potentiated by both PEA (specific substrate for MAO-B) and deprenyl (selective inhibitor of this enzyme). The analgesic effect of phosphoramidon was probably due to an increase in endogenous enkephalins and the consequent activation of opioid receptors sensitive to naloxone and naltrexone (Chaillet et al. 1983; Rupreht et al. 1983). We suggest that excess of PEA facilitates the analgesic action of enkephalins at the synaptic sites. This is in accordance with reports indicating that MAO-B inhibition and/or excess PEA potentiate the pharmacological effects of exogenously administered opiates/opioid peptides (Fuentes et al. 1977; Garzon et al. 1980; Ukponmwan et al. 1983).

The mechanism by which MAO-B inhibition or excess PEA potentiates the analgesic effect of endogenously released enkephalins is not clear. It is possible that PEA enhances the interaction between opiates and their receptors (Fuentes et al. 1977). Thus, the MAO-B system may be an important regulator of the activity of opioids at the synaptic site in animals allowed undisturbed sleep.

The described facilitatory action of MAO-B inhibition on enkephalinergic transmission in animals allowed undisturbed sleep might be of relevance not only in the physiology of nociception, but also in human disorders in which the alterations in MAO-B have been reported. For example, it has been demonstrated that endogenous depression is associated with a decrease in brain PEA levels (Wolf and Mosnaim 1983), whereas an increase in MAO-B activity is associated with the aging process (Benedetti and Keane 1980). In such cases an alteration in MAO-B activity and PEA levels could modify the analgesic effects of endogenous opioid peptides.

However, the results of this study indicate that possible alterations in the bioavailability of the MAO-B substrate, PEA, may not play an essential role in the failure of phosphoramidon to induce analgesia in REM sleep deprived rats. This statement is based on the fact that deprenyl and/or PEA did not alter the inhibitory effect of REMSD on phosphoramidon-induced analgesia.

The basal pain threshold was lowered in rats deprived of REM sleep. This is in accordance with a previous study in which, using noxious electric shock to assess pain sensitivity, it was established that REMSD decreased the pain threshold (Hicks et al. 1979). The reason for the reduction in the pain threshold in REM sleep deprived rats is not clear. It might be due to the already suggested functional insufficiency of enkephalinergic/endorphinergic system during REMSD (Ukponmwan and Dzoljic 1984b; Ukponmwan et al. 1985) since opioid peptides play an important role in the regulation of the pain threshold (Basbaum and Fields 1984).

A functional insufficiency of an opioid system during REMSD (Ukponmwan et al. 1985) might partly explain why REM sleep curtailment is beneficial in treating some forms of depression (Vogel et al. 1980), since an increased opioid activity and corresponding decrease in pain sensitivity have been observed in this affective disorder (Risch 1982; Pickar et al. 1982; Davis et al. 1979).

Further clinical experiments are necessary to clarify the roles of MAO-B and the enkephalinergic/endorphinergic systems in the regulation of the pain threshold in human diseases.

Acknowledgement. The authors wish the express their gratitude to Dr. G. Elliott, Dept of Pharmacology, Erasmus University of Rotterdam for his linguistic revision of the text.

References

Basbaum AI, Fields HL (1984) Endogenous pain control systems: brain spinal patways and endorphin circuitry. Ann Rev Neurosci 7:309–338

Benedetti MS, Keane PE (1980) Differential changes in monoamine oxidase A and B activity in the aging rat brain. J Neurochem 35:1026–1032

Boden R, Botting R, Coulson P, Spanswick G (1984) Effect of nonselective and selective inhibitors of monoamine oxidases A and B on pethidine toxicity in mice. Br J Pharmacol 82:151–154

Chaillet P, Coulaud A, Fournie-Zaluski MC, Gacel G, Roques BP, Costentin J (1983) Pain control by endogenous enkephalins is mediated by mu opioid receptors. Life Sci 33:685–688

Davis GC, Buchsbaum MS, Bunney WE (1979) Analgesia to painful stimuli in affective illness. Am J Psychiatr 136:1148–1151

Dourish CT, Cooper SJ (1984) Potentiation of total horizontal activity and ambulation in rats treated with combinations of β-phenylethylamine and naloxone. Neuropharmacology 23:1059–1064

Fuentes JA, Garzon J, Del Rio J (1977) Potentiation of morphine analgesia in mice after inhibition of brain type-B monoamine oxidase. Neuropharmacology 16:857–862

Garzon J, Moratalla R, Del Rio J (1980) Potentiation of the analgesia induced in rats by morphine or [D-Ala^2]-met-enkephalinamide after inhibition of brain type B monoamine oxidase: the role of phenylethylamine. Neuropharmacology 19:723–729

Garrick NA, Murphy DL (1980) Species differences in the deamination of dopamine and other substrates for monoamine oxidase in the brain. Psychopharmacology 72:27–33

Hicks RA, Coleman DD, Ferrante F, Sahatjian M, Hawkins J (1979) Pain thresholds in rate during recovery from REM sleep deprivation. Percept Mot Skills 48:687–690

Hudgin RL, Charleson SE, Zimmerman M, Mumford R, Wood PL (1981) Enkephalinase: selective peptide inhibitors. Life Sci 29:2593–2601

Iwamoto ET, Ho IK (1972) The effects of MAO inhibition on morphine analgesia in mice. Fed Proc 31:504

Jounela AJ, Mattila MJ, Knoll J (1977) Interaction of selective inhibitors of monoamine oxidase with pethidine in rabbits. Biochem Pharmacol 26:806–808

Kubota K, Matsuoka Y, Sakuma M, Satoh S, Uruno T, Sunagane N (1982) Characteristic of analgesia induced by noncatecholic phenylethylamines in mice. Life Sci 31:1221–1224

Mann J, Gershon S (1980) L-Deprenyl, a selective monoamine oxidase type-B inhibitor in endogenous depression. Life Sci 26:877–882

Mendelson WB, Guthrie RD, Frederick G, Wyatt RJ (1974) The flower pot technique of rapid eye movement (REM) sleep deprivation. Pharmacol Biochem Behav 2:553–556

Pickar D, Cohen MR, Naber D, Cohen RM (1982) Clinical studies of the endogenous opioid system. Biol Psychiatr 17:1243–1276

Randall OL, Selitto JJ (1957) A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn 111:409–419

Risch S (1982) β-Endorphin hypersecretion in depression: possible cholinergic mechanisms. Biol Psychiatr 17:1071–1079

Rupreht J, Ukponmwan OE, Admiraal PV, Dzoljic MR (1983) Effect of phosphoramidon – a selective enkephalinase inhibitor – on nociception and behaviour. Neurosci Letts 41:331–335

Saxena PR (1985) An interactive computer programme for data management and parametric and non-parametric statistical analysis. Proc Br Pharmacol Soc Edinburgh Abstr D3

Steel RGD, Torrie JH (1980) Principles and procedures of statistics, biometrical approach, McGraw-Hill Kogakusha Ltd, Tokyo

Taylor DC (1962) Alarming reaction to pethidine in patients on phenelzine. Lancet ii 401–402

Ukponmwan OE, Poel-Heisterkamp AL van den, Haffmans J, Dzoljic MR (1983) MAO-B inhibitor deprenyl and β-phenylethylamine potentiate [D-Ala^2]-met-enkephalinamide-induced seizures. Naunyn-Schmiedeberg's Arch Pharmacol 322:38–41

Ukponmwan OE, Rupreht J, Dzoljic MR (1984a) REM sleep deprivation decreases the antinociceptive property of enkephalinase inhibition, morphine and cold-water stress. Gen Pharmacol 15:255–258

Ukponmwan OE, Dzoljic MR (1984b) Enkephalinase inhibition antagonizes the increased susceptibility to seizure induced by REM sleep deprivation. Psychopharmacology 83:229–232

Ukponmwan OE, Poel-Heisterkamp AL van den, Dzoljic MR (1985) REM sleep deprivation decreases the grooming and shaking behaviour induced by enkephalinase inhibitor or opiate withdrawal. Pharmacol Biochem Behav 23:385–389

Vogel GW, Vogel F, McAbee RS, Thurmond AJ (1980) Improvement of depression by REM sleep deprivation. Arch Gen Psychiat 37:247–253

Wolf ME, Mosnaim AD (1983) Phenylethylamine in neuropsychiatric disorders. Gen Pharmacol 14:385–390

Wu PH, Boulton AA (1975) Metabolism, distribution and disappearance of injected β-phenylethylamine in the rat. Can J Biochem 53:42–50

Yang H-YT, Neff NH (1974) The monoamine oxidases of brain: selective inhibition with drugs and the consequences for the metabolism of the biogenic amines. J Pharmacol Exp Ther 189:733–740

Received September 2, 1985/Accepted December 3, 1985