ELSEVIER

European Journal of Pharmacology 284 (1995) 191–193

ejp

Short communication

Potentiation of morphine analgesic action in mice by β-carotene

Nathar W. Penn *

The Nathan S. Kline Institute for Psychiatric Research, Center for Neurochemistry, Orangeburg, NY 10962, USA

Received 1 June 1995; revised 6 July 1995; accepted 7 July 1995

Abstract

β-Carotene was found to potentiate morphine analgesia in hot plate experiments using mice. Response time was increased by about 100%, and the duration of analgesia was markedly prolonged. These features of β-carotene were a function of the intact molecule, although the closely related compound, α-carotene, also exhibited this property. β-Carotene alone had no effect on the nociceptive response. Other compounds with related structures, metabolic products, or anti-oxidants neither augmented nor antagonized morphine action.

Keywords: β-Carotene; Morphine; Analgesia

1. Introduction

During the study of one-carbon compound metabolism in this laboratory (work in progress), it was observed that the effect of morphine could be potentiated by certain compounds normally present in the mammalian cell. β-Carotene was found to be the active agent. Since our data suggested that morphine might intervene in a methyl transfer system, it was necessary to determine whether β-carotene was a specific factor. Therefore, other related compounds were also tested.

2. Materials and methods

Male or female mice of the BALC/cBYj strain weighing 21 ± 2 g were obtained from the NKI animal colony, Orangeburg, NY, USA. Morphine sulfate, USP, was purchased from Wyeth (Philadelphia, PA, USA); α- and β-carotenes, lycopene, retinol, retinoic acid, β-ionone, *dl*-α-tocopherol, Na ascorbate and propylene glycol of the highest available purity were from the Sigma Chemical Co. (St. Louis, MO, USA).

The procedure was a modification of that employed by Welch and Dewey (1990). A thermostated water bath, holding temperature at 57 ± 0.5°C (Precision Scientific, Chicago, IL, USA) in a draft-free room was used as the heat source. Water was added to the same level for all trials and a large beaker in which the mouse was to be placed, was set in the bath. The same beaker was used each time. The temperature in the beaker was set at 57.5°C with a thermometer resting on the bottom of the dry beaker after thermal equilibrium was reached. Under these conditions, air temperature 3 cm above the bottom of the beaker was 46.5°C. The test was conducted with the thermometer in contact with the bottom of the beaker. Temperature was checked at the beginning and end of each trial.

All injections were given i.p. The carotenes and lycopene were gently homogenized by hand in propylene glycol to give a final concentration of 20 mg/ml using a Teflon-glass homogenizer and avoiding the inclusion of air. A No. 20 needle was used for administration of particulates and resuspension was found to be advisable before each injection. Retinoic acid was dissolved in 0.5 M $NaHCO_3$. Retinol was dissolved in ethanol and then suspended by addition of saline to give a final 3% v/v concentration of the ethanol. β-Ionone and *dl*-α-tocopherol were injected directly. Light-sensitive materials were administered promptly in the absence of the direct illumination. Controls for the various injections were carried out on the same day. Morphine sulfate alone, or with the appropriate compounds, was given at zero time. Mice were tested 1 or 3 h later. Morphine sulfate, 7.14 mg/kg, was the standard dosage for morphine throughout these experi-

* Tel. (914) 365-2000 ext. 1314, fax (914) 359-7029.

SSDI 0014-2999(95)00412-2

ments. One or three hours after injection the animal was placed in the beaker and the time required for appearance of discomfort, such as paw-licking, sudden accelerated movement, or leaps was determined, using a stop-watch. The cut-off time was 25 s. As soon as any symptom was noted, the mouse was promptly removed from the beaker. Data were analyzed by the Mann-Whitney *U*-test and the Kruskal-Wallace analysis of variance. Some of the data were also examined with an analysis of variance (parametric).

3. Results

At a dosage of 38.1 mg/kg of β-carotene, the analgesic effect of morphine was increased by 110% at 1 h (Table 1). Response time was dose-dependent (Fig. 1). In the absence of morphine, β-carotene at the optimum dosage of 38.1 mg/kg had no analgesic effect at either 1 or 3 h (Table 1). β-Carotene alone had no effect on response time over the dosage range of 7.14 mg/kg to 57.1 mg/kg used in these trials (Fig. 1). At the volumes employed, none of the solvents or suspending vehicles had any effect on the analgesic action of morphine or on the nociceptive response in non-morphinized animals (data not shown).

There was no significant difference between the action of α- and β-carotene. Retinol and retinoic acid,

Table 1
Effect of carotenes and related compounds on morphine analgesic action in mice

	Dosage (mg/kg)	Response time [a]	
		1 h	3 h
Normal		3.0±0.1 (8) [b]	
Morphine sulfate	7.1	8.2±0.2 (7) [c]	3.5±0.3 (8)
β-Carotene	38.1	3.6±0.4 (8)	3.2±0.2 (8)
+ morphine		17.0±0.2 (10) [d]	7.7±0.4 (8) [e]
α-Carotene + morphine	38.1	17.8±0.6 (8) [d]	7.9±0.6 (8) [e]
Lycopene + morphine	38.1	9.4±0.6 (8)	
Retinol + morphine	19.1	8.0±1.2 (10)	
	38.1	8.6±0.4 (9)	
Retinoic acid	19.1	8.7±0.5 (9)	
+ morphine	38.1	8.4±0.8 (9)	
β-Ionone + morphine	14.2	7.3±0.5 (8)	
	28.4	7.9±0.3 (8)	
dl-α-Tocopherol	14.2	8.7±0.5 (10)	
+ morphine	33.3	9.0±0.6 (10)	
	47.6	8.0±0.4 (10)	
Na ascorbate + morphine	28.4	8.9±0.5 (8)	
	14.2	9.8±0.5 (8)	

[a] Values are expressed as means in seconds ± S.E.M. [b] Number of animals. [c] Response time differs from normal value, $P < 0.01$. [d] Differs from all other values, $P < 0.01$. [e] Differs from other 3 h values, $P < 0.01$. There is no significant difference between the effect of morphine given alone, or with lycopene and the non-carotenoid compounds.

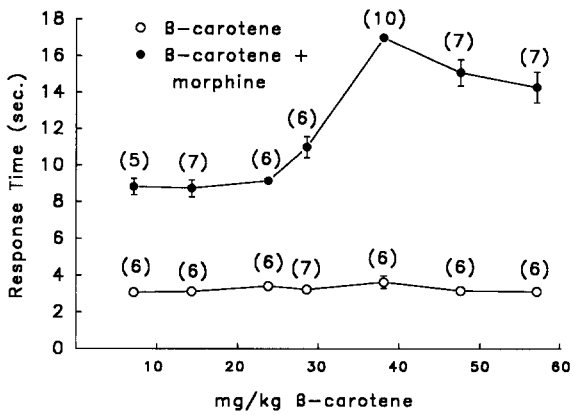

Fig. 1. Effect of β-carotene dosage on the analgesic response in mice in the absence and presence of 7.14 mg morphine sulfate/kg.

and other related compounds, such as lycopene and β-ionone, had no effect on the response to morphine (Table 1). Compounds considered to be antioxidants or free radical scavengers, such as *dl*-α-tocopherol and Na ascorbate (Perry et al., 1985) did not alter the nociceptive response in the presence of morphine (Table 1).

Potentiation of the morphine effect by β-carotene was also evident from the prolongation of analgesia. At 3 h the analgesia due to morphine alone was no longer detectable, based on a comparison of response times of untreated normal mice, 3.0 s, with morphinized animals, 3.5 s ($P > 0.05$). However, mice given morphine and β-carotene still exhibited a significantly increased response time, over 100%, at 3 h, 7.7 s compared with either the normal or the morphinized mice (Table 1).

4. Discussion

Although a variety of components native to the cell, such as Ca^{2+} (Kakunaga et al., 1966), L-dihydroxyphenylalanine (Vander and Spoerlin, 1972), prostaglandin E_1 (Ehrenpreis et al., 1973), adenine compounds (Gourley and Beckner, 1973; Ho et al., 1973), and deoxycytidine (Penn, 1977), can modify morphine effects, these materials are generally neuroactive. To a greater or lesser degree, all antagonize opiate action. The carotenes, however, are unusual in that they augment morphine analgesia without exhibiting any independent neuroactive properties. This increase in morphine effectiveness also extends to 3 h, by which time the animals given only morphine no longer show analgesia, while those given β-carotene with morphine are still significantly affected (Table 1).

The well-established functions of β-carotene as an antioxidant (Perry et al., 1985), and as a precursor of retinol (Ganguly and Murthy, 1967), do not appear to

be related to this effect on opiate action. Morphine is primarily detoxified by conjugation with glucuronic acid (Jaffe, 1965), not by oxidation. Furthermore, other antioxidants such as Na ascorbate and *dl*-α-tocopherol tested in a range of molecular concentrations above and below that of the optimal level of β-carotene, do not augment the analgesia. The non-specific inhibition of oxidative reactions therefore does not account for our observations.

Neither retinol nor retinoic acid are known to potentiate or antagonize morphine action. In our tests of these compounds at two different concentrations, no significant effects were obtained (Table 1). Conversion of β-carotene to the retinoids therefore does not appear to be responsible for this phenomenon.

It is possible that carotenes alter the blood-brain barrier to permit a more rapid entry of morphine into the brain. However, uptake of morphine by brain slices does not require an active transport process (Teller et al., 1974), and uptake reaches its maximal level in the brain of the intact animal 1–2 min after intravenous injection (Oldendorf et al., 1972). A considerable increase in the rate of entry would therefore, by itself, not account for the 100% increase in analgesic effect at 1 and 3 h. Should carotene administration result in the formation of a lipid depot or compartment for morphine retention, these observations might be explained. By itself, such a mechanism would not however be sufficient to explain our results, since it does not permit interpretation of the sharp maximum observed as carotene dosage reaches its optimal level of 38.1 mg/kg (Fig. 1). In addition, related lipid structures such as lycopene, β-ionone, retinol, *dl*-α-tocopherol do not give similar effects.

Conceivably, at the levels employed, β-carotene may serve as an accessory factor stabilizing a complex of the morphine molecule at its binding site. However, the existence of a variety of components which exhibit antagonism to morphine action raises the possibility that β-carotene may also intervene in biochemical sequelae to the initial opiate binding. It should be noted that there is a very high degree of specificity required for the potentiation we have found. Only α- and β-carotene exhibit this activity.

In initial trials, those compounds which did not affect morphine action at 1 h were also ineffective at 3 h (data not shown).

Acknowledgement

The author wishes to thank Dr. Anthony Badalamenti, of the Nathan S. Kline Institute, for the statistical analyses of these data.

References

Ehrenpreis, S., J. Greenberg and S. Belman, 1973, Prostaglandins reverse inhibition of electrically-induced contractions of guinea pig ileum by morphine, indomethacin and acetylsalicylic acid, Nature New Biol. 245, 280.

Ganguly, J. and S.K. Murthy, 1967, Biogenesis of vitamin A and carotene, in: The Vitamins, 2nd edn., eds. W.H. Sebrell Jr. and R.S. Harris (Academic Press, New York) p. 139.

Gourley, D.R.H. and S.K. Beckner, 1973, Antagonism of morphine analgesia by adenine, adenosine, and adenine nucleotides, Proc. Soc. Exp. Biol. Med. 144, 774.

Ho, I.K., H.H. Loh and E.L. Way, 1973, Cyclic adenosine monophosphate antagonism of morphine analgesia, J. Pharmacol. Exp. Ther. 185, 336.

Jaffe, J.H., 1965, Drugs acting on the central nervous system: narcotic analgesics, in: The Pharmacological Basis of Therapeutics, 3rd edn., eds. L.S. Goodman and A. Gilman (Macmillan Co., New York) p. 259.

Kakunaga, T., H. Kaneto and K. Hano, 1966, Pharmacologic studies on analgesics. VII. Significance of the calcium ion in morphine analgesia, J. Pharmacol. Exp. Ther. 153, 134.

Oldendorf, W.H., S. Hyman, L. Braun and S.Z. Oldendorf, 1972, Blood-brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection, Science 178, 984.

Penn, N.W., 1977, Deoxycytidine: a morphine antagonist, Arch. Int. Pharmacodyn. Ther. 223, 145.

Perry, T.L., V.W. Yong, R.M. Clavier, K. Jones, J.M. Wright, J.G. Foulks and R.A. Wall, 1985, Partial protection from the dopaminergic neurotoxin *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by four different antioxidants in the mouse, Neurosci. Lett. 60, 109.

Teller, D.N., T. DeGuzman and A. Lajtha, 1974, The mode of morphine uptake into brain slices, Brain Res. 77, 121.

Vander, W.C. and M.T. Spoerlin, 1972, Antagonism by dopa of morphine analgesia. A hypothesis for morphine tolerance, Res. Commun. Chem. Pathol. Pharm. 3, 37.

Welch, S.P. and W.L. Dewey, 1990, The activity of several peptide fragments of parathyroid hormone, alone and in combination with salmon calcitonin and morphine, in antinociceptive tests in the mouse, J. Pharmacol. Exp. Ther. 252, 140.