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MUSCIMOL ANTAGONISM OF MORPHINE ANALGESIA IN RATS

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1 Muscimol, a γ-aminobutyric acid (GABA) receptor agonist, when injected intraventricularly antagonizes the antinociceptive effect of morphine given either subcutaneously or intraventricularly. The antagonistic effect of muscimol on morphine analgesia appears to be linearly related.

2 This finding provides support for the view that a GABA-ergic system is involved in morphine analgesia.

Introduction

It has recently been suggested that γ-aminobutyric acid (GABA) is involved in morphine analgesia. Acute administration of morphine has been reported to induce significant increases of GABA content and L-glutamate decarboxylase (GAD) activity in the dorsal horn of spinal cord and in the thalamus of rats (Kuriyama & Yoneda, 1978).

In addition to this biochemical data it has been shown that aminooxyacetic acid (AOAA), which elevates brain levels of GABA by slowing its destruction (Gottesfeld, Kelly & Renaud, 1972), potentiates the morphine antinociceptive action in mice (Yoneda, Takashima & Kuriyama, 1976). In contrast to this, Ho, Loh & Way (1976) found that the administration of AOAA and 2,4-diaminobutyric acid, an inhibitor of GABA uptake in neurones (Schon & Kelly, 1974), antagonized morphine analgesia in mice.

Recently Biggio, Della Bella, Frigeni & Guidotti (1977) have shown that muscimol, a GABA agonist (Scheel-Krüger, Arnt & Magelund, 1977), injected intravenously in small doses, enhanced the analgesic effect of morphine in rats and mice. On the other hand Christensen, Arnt & Scheel-Krüger (1978) found that the same dose of muscimol induced only a very weak increase in the morphine analgesia measured in the hot plate test, while no effect on morphine analgesia was observed in the wire grid test.

It has been claimed that muscimol slowly crosses the blood brain barrier and/or that a muscimol metabolite may mediate the effects ascribed to this drug when injected intravenously (Costa, 1977).

To examine these possibilities the effect of intraventricular injection of muscimol has been investigated on morphine analgesia in rats.

Methods

Sprague–Dawley male rats of Charles River strain weighing 110 to 120 g were used. They were fed with a standard laboratory diet and given tap water. Standard living conditions included a room temperature of 22 ± 1°C. Food was withdrawn 12 h before the beginning of experiments, whereas access to water was permitted throughout.

Antinociceptive activity was measured with the 'tail flick' method according to D'Amour & Smith (1941).

The 'reaction time' to heat was measured in tenths of a second: in basal conditions the typical nociceptive response was obtained after 3.5 to 4.5 s. Measurement was not continued for longer than 8.0 s in order to prevent a tail scald.

The experiment was carried out as follows: all animals were implanted, under barbiturate anesthesia (Nembutal 30 mg/kg) with a permanent polyethylene cannula (PE 10 i.d. 0.001 in, o.d. 0.025 in) into both lateral cerebral ventricles, according to the method of Altaffer, De Balbian Werster, Hall, Long & D'Encarnacao (1970). Animals were placed in individual cages. After seven days they reached a body weight of 160 to 170 g. On the seventh day the reaction time of all animals was measured. Then, without anaesthesia, the animals were injected into each ventricle with various dose levels of muscimol (62.5; 125; 250 ng) dissolved in 10 μl saline immediately before use. Control animals received an equal volume of saline (0.9% w/v NaCl solution). Morphine hydrochloride was administered 30 min after muscimol, either subcutaneously in a dose of 2.5 mg/kg, or intraventricularly at a dose of 0.5 μg/ventricle. These doses of morphine were chosen because preliminary experiments showed they caused a two fold increase in the

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reaction time. Statistical evaluation of the data was carried out by means of the analysis of variance. Comparisons among groups were done by means of a multiple range test, according to Kramer (1956) owing to the uneven number of rats in each of the various groups, unless indicated otherwise in the table.

The method of least squares was used to calculate the best-fit curves. In addition an analysis of variance was performed in order to evaluate the linear regression.

Results

Tables 1 and 2 show the increase in the tail flick reaction time with increasing doses of morphine injected subcutaneously or intraventricularly. These experiments were performed to select the most suitable dose of morphine for studying the effect of muscimol on morphine analgesia. With respect to preliminary experiments in which muscimol was shown to reduce the morphine antinociceptive effect, it was decided to use doses of morphine which increased the tail flick reaction time about two fold.

Muscimol 250 ng/ventricle *per se* induced no significant change in the tail flick reaction time as compared to the control group receiving saline–saline injections only, but strongly suppressed the antinociceptive effect of morphine injected both subcutaneously and intraventricularly (morphine 2.5 mg/kg s.c., 0.5 μg/ventricle) (Figures 1 and 2). This effect of muscimol was linearly-related to the log of the dose administered at 30, 60 and 90 min after morphine injection (Figure 3).

The lower dose of muscimol (62.5 ng/ventricle) did not suppress the morphine analgesia during the time intervals 0 to 120 min after morphine injection (2.5 mg/kg s.c.), while muscimol (125 and 250 ng/ventricle)

Table 1 Antinociceptive effect in rats treated subcutaneously with various doses of morphine

			Reaction time (s)				
				After morphine			
Treatment	*No. of animals*	*Dose (mg/kg)*	*Before morphine*	*30 min*	*60 min*	*90 min*	*120 min*
Saline	10	—	4.060 ± 0.075	4.100 ± 0.124	3.995 ± 0.055	4.105 ± 0.085	4.085 ± 0.049
Morphine	10	0.625	4.180 ± 0.057	5.955 ± 0.252**	4.727 ± 0.080**	4.222 ± 0.053	4.096 ± 0.048
Morphine	10	1.25	3.876 ± 0.054	5.624 ± 0.202**	5.852 ± 0.179**	4.978 ± 0.049**	4.522 ± 0.076**
Morphine	10	2.50	4.104 ± 0.077	7.246 ± 0.300**	7.688 ± 0.214**	6.814 ± 0.326**	5.750 ± 0.110**
Morphine	10	5.00	3.990 ± 0.076	8.000 ± 0.000**	8.000 ± 0.000**	8.000 ± 0.000**	8.000 ± 0.000**

The statistical significances of the differences between morphine and saline were calculated by Dunnet's test (1964) (** $P \leqslant 0.01$).
Values are means ± s.e. mean.

Table 2 Antinociceptive effect in rats treated intracerebroventricularly with various doses of morphine

			Reaction time (s)						
				After morphine					
Treatment	*No. of animals*	*Dose (μg/ventricle)*	*Before morphine*	*10 min*	*20 min*	*30 min*	*60 min*	*90 min*	*120 min*
Saline	6	—	4.265 ± 0.183	4.335 ± 0.225	4.325 ± 0.230	4.210 ± 0.195	4.325 ± 0.245	4.245 ± 0.272	4.433 ± 0.360
Morphine	6	0.25	4.220 ± 0.198	5.300 ± * 0.243	5.640 ± ** 0.331	5.060 ± * 0.209	4.600 ± 0.239	4.300 ± 0.307	4.020 ± 0.150
Morphine	6	0.50	4.320 ± 0.246	6.400 ± ** 0.511	7.460 ± ** 0.340	7.400 ± ** 0.255	6.540 ± ** 0.346	5.720 ± ** 0.162	4.660 ± 0.462
Morphine	6	1.00	4.180 ± 0.334	5.540 ± ** 0.296	6.980 ± ** 0.487	7.700 ± ** 0.300	7.340 ± ** 0.660	6.360 ± ** 0.874	5.440 ± 0.970
Morphine	6	2.00	4.120 ± 0.149	6.140 ± * 0.535	7.640 ± ** 0.475	7.800 ± ** 0.379	7.700 ± ** 0.700	7.480 ± ** 0.585	5.980 ± 0.682

The statistical significance of the differences between morphine and saline were calculated by Dunnet's test (* $P \leqslant 0.05$, ** $P \leqslant 0.01$).
Values are means ± s.e. mean.

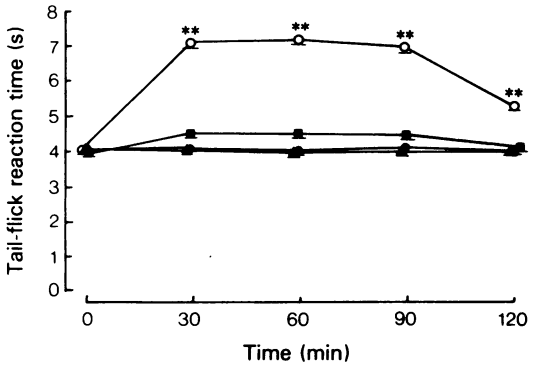

Figure 1 Antinociceptive effect of morphine (2.5 mg/kg s.c.) in rats implanted with a cannula in each lateral brain ventricle after treatment with saline or with muscimol (250 ng/each ventricle). (●) Saline + saline; (▲) muscimol + saline; (○) saline + morphine; (■) muscimol + morphine. Mean values are given; vertical lines show s.e. mean. The statistical significances (** $P < 0.01$) calculated by the Kramer test refer to differences from the saline group.

significantly antagonized the morphine analgesia at 60 and 90 min respectively (Table 3).

Discussion

In a previous study the intravenous injection of muscimol has been reported to potentiate the anti-nociceptive effect of morphine in rats and mice (Biggio *et al.*, 1977) while in other studies no effect was found in mice (Christensen *et al.*, 1978). In contrast, our results demonstrate that centrally administered muscimol strongly antagonizes the antinociceptive activity of morphine given either subcutaneously or intraventricularly in rats.

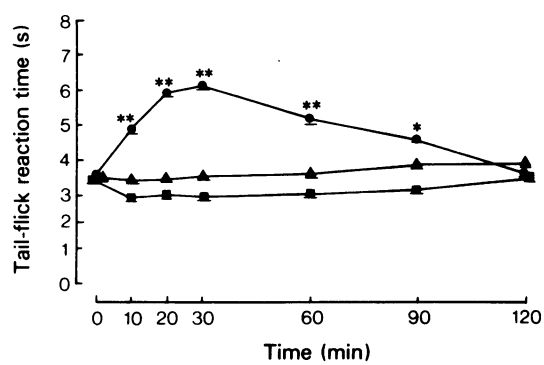

Figure 2 Antinociceptive effect of morphine (0.5 μg/ventricle) injected into a cannula implanted in each lateral brain ventricle after treatment with saline or muscimol (250 ng/ventricle). (▲) Muscimol + saline; (●) saline + morphine; (■) muscimol + morphine. Mean values are given; vertical lines shows s.e. mean. The statistical significances (* $P \leq 0.05$, ** $P \leq 0.01$) calculated by Kramer test refer to the differences from the muscimol-treated group.

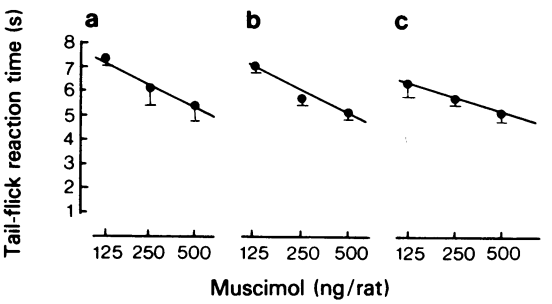

Figure 3 Effect of muscimol on morphine analgesia. Muscimol (125, 250, 500 ng/rat) was administered intraventricularly 30 min before morphine (2.5 mg/kg s.c.). (a) Semilogarithmic plot of the increasing dose of muscimol against the tail flick reaction time measured 30 min after morphine injection; (b) measured 60 min after morphine; (c) measured 90 min after morphine. Each point is the mean result from six rats; vertical lines show s.e. mean.

It may be that the opposing results obtained in rats following the intraventricular or intravenous administration on morphine analgesia might reflect differences in muscimol distribution within the central nervous system. In fact it has been claimed that the population of receptors bound by muscimol after peripheral administration may differ qualitatively from that accessible to intraventricular administration of the drug (Snodgrass, 1978).

The possibility that muscimol produces its effect by acting directly on brain areas associated with opiate receptors should be considered. This suggestion is supported by biochemical studies which have demonstrated that glutamate decarboxylase activity and GABA-ergic neurones are located in the periaqueductal gray matter and in the raphe magnus nucleus (Belin, Aguera, Tappaz & Pujol, 1978), the principal supraspinal sites where morphine is thought to act to produce antinociception (Yaksh, Yeung & Rudy, 1976; Yaksh, Plant & Rudy, 1977). Other brain structures may be also involved in the observed effect of muscimol on morphine analgesia. Muscimol causes an inhibition of the firing rate of the cells of the pars reticulata of the substantia nigra (Walters & Lakoski, 1978) from which there is a projection of a non-dopaminergic pathway to the periaqueductal gray (Grofova, Ottersen & Rinvik, 1978). Muscimol also increases the firing rate of dopaminergic neurones projecting from the pars compacta of the substantia nigra to the striatum (Walters & Lakoski, 1978) and pro-

Table 3 Antinociceptive effect of morphine (2.5 mg/kg s.c.) in rats pretreated with various doses of muscimol intracerebroventricularly.

Treatment	No. of animals	Muscimol dose (ng/ventricle)	Reaction time (s) Before muscimol and morphine	After morphine 30 min	60 min	90 min	120 min
A Saline + morphine	6	0	4.283 ± 0.127	7.717 ± 0.200	7.417 ± 0.524	6.900 ± 0.408	4.850 ± 0.358
B Muscimol + morphine	6	62.5	4.417 ± 0.156	7.333 ± 0.803	7.033 ± 0.329	6.266 ± 0.584	5.300 ± 0.478
C Muscimol + morphine	6	125	4.300 ± 0.200	5.883 ± 0.360	5.717 ± 0.341	5.617 ± 0.176	4.967 ± 0.206
D Muscimol + morphine	6	250	4.517 ± 0.075	5.533 ± 0.518	5.117 ± 0.326	5.033 ± 0.307	4.566 ± 0.095
A → B			NS	NS	NS	NS	NS
A → C			NS	HS	S	NS	NS
A → D			NS	HS	HS	S	NS

NS = $P \geqslant 0.05$ S = $P \leqslant 0.05$ HS = $P \leqslant 0.01$.
Significance of difference among groups was calculated by Dunnet's test.
Values are means ± s.e. mean.

motes a stimulation of [^{3}H]dopamine release in the caudate nucleus after direct application of the drug into the substantia nigra (Chéramy, Nieoullon & Glowinski, 1978).

A similar increase of dopaminergic activity following muscimol administration could occur in brain areas implicated in morphine analgesia. Since the antinociceptive effect of morphine is antagonized by dopaminergic stimulation (Vanderwende & Spoerlein, 1973; Tulunay, Sparber & Takemori, 1975) and potentiated by dopaminergic blockade (Tulanay & Takemori, 1974; Eidelberger & Espamer, 1975; Paalzow & Paalzow, 1975) it is possible that muscimol could produce its effect by modulating the dopaminergic system.

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