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Influence of different concentrations of nalorphine on the *in vitro* N-demethylation of morphine

Influencia de distintas concentraciones de nalorfina sobre la N-desmetilación *in vitro* de la morfina

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The influence of different concentrations of nalorphine on the *in vitro* N-demethylation of morphine by liver homogenates was studied. Rat liver supernatant (9.000 × g) was incubated with morphine and nalorphine in ratios of 1:1, 1:0.1 or 1:0.01. Morphine concentration was 1.3×10^{-3}M in all experiments. In ratio 1:1, nalorphine inhibited morphine N-demethylation by 70% after 120 minutes of incubation; in lower concentration (ratio 1:0.1) the inhibition was only 10%. In contradistinction in the lowest concentration (ratio 1:0.01) malorphine enhanced the N-demethylation of morphine by 25%.

NALORPHINE MORPHINE *IN VITRO* N-DEMETHYLATION

The results reported in the literature about the effect of nalorphine on the enzymatic N-demethylation of morphine either *in vitro* or *in vivo* appear controversial.

Concerning *in vitro* studies performed by incubation of liver homogenates, Axelrod and Cochin (1) reported that nalorphine 4×10^{-4}M inhibited by 75% the N-demethylation of morphine 1×10^{-3}M. In similar experiments Leadbeater and Davies (2) observed a mixture of competitive and non competitive antagonism of nalorphine 3×10^{-4}M on N-demethylation of morphine (around 2×10^{-4}M). In contradistinction. Tampier *et al.* have reported (3) that nalorphine $1,5 \times 10^{-6}$M enhanced N-demethylation of morphine 2×10^{-4}M by rat liver homogenates.

As far as *in vivo* experiments are concerned, Minegishi *et al.* (4) observed that in rats the pretreatment with nalorphine (100 mg/kg) resulted in an inhibition of morphine (100 mg/kg) N-demethylation. The results reported by Tampier *et al.* (3) show that liver homogenates of rats pretreated with 20 mg/kg of nalorphine exhibited an N-demethylation of morphine significantly higher than untreated controls.

Since the ratio morphine: nalorphine was widely different in the reported experiments it was pertinent to study the influence of graded concentrations of nalorphine on N-demethylation of morphine under equivalent experimental conditions. The present paper reports on the results of experiments in which rat liver homogenates were incubated with morphine and nalorphine in concentration ratios of 1:1, 1:0.1 and 1:0.01. Morphine N-demethylation was estimated by the formaldehyde content of the medium at different times of incubation.

MATERIAL AND METHODS

Adult male albino rats of our colony were killed by cutting the abdominal aorta under urethane anesthesia (1.1 g/kg i.p.). Inmediately after, the liver was removed and a 20% homogenate in 0.1 M KH_2PO_4, pH 7.4, containing 1% glucose was prepared. Homogenates were centrifuged at 9,000 × g for 30 minutes at 2°C, and an aliquot of the supernatant, equivalent to 1 g of tissue, was added to flasks containing 1 ml of any of the following alkaloids concentrations: a) Morphine HCl 7.8 × 10^{-3}M; b) Morphine HCl 7.8 × 10^{-3}M plus Nalorphine HBr 7.8 × 10^{-3}M; c) Morphine HCl 7.8 × 10^{-3}M plus Nalorphine HBr 7.8 × 10^{-4}M and d) Morphine HCl 7.8 × 10^{-3}M plus Nalorphine HBr 7.8 × 10^{-5}M. After 5 minutes incubation at 36°C in a Dubnoff bath, 1 ml of a solution containing the following substances: NADP 0.3 μmole; Nicotinamide, 60 μmole; Semicarbazide, 100 μmole and $MgCl_2$ 25 μmole was added to each fiask. Final volume of 6 ml was completed with phosphate buffer, and the solutions were incubated in same conditions. Samples of 1 ml were taken before incubation of the mixture and at 5, 10, 15, 60 and 120 minutes of incubation. In each sample the reaction was stopped by adding 0.5 ml 20% trichloroacetic acid. After centrifuged, the amount of formaldehyde present was measured by the method described by Nash (5). Known amounts of formaldehyde carried through the incubation and assay procedures served as standards.

RESULTS AND DISCUSSION

The results summarized in table I show that when nalorphine was added to the incubation media in the same concentration as morphine, the N-demethylation of the last alkaloid was significantly blocked in such a way that at 120 minutes it decreased by about 70%. When the concentration of nalorphine was one tenth of that of morphine, the blocking of morphine N-demethylation was lower, reaching at 120 minutes only a 10% decrease. In contradistinction, when the concentration of nalorphine was only one hundredth of that of morphine a significant enhancement of morphine N-demethylation (25% at 120 minutes of incubation) was observed.

These results explain the apparent contradictory results reported in the literature, since they show that the effect of nalorphine on morphine N-demethylation was different according to the concentration ratio of the two alkaloids: at the same concentration of morphine, nalorphine, acted as antagonist, while at a concentration equivalent to one hundredth of that of morphine it enhanced the enzymatic activity.

In relation with the interaction of both alkaloids, Lasagna and Beecher (6) observed that the analgesic effect of a mixture of 10 mg of morphine and 2 mg of nalorphine was not significantly different from that of 10 mg of morphine alone in man. Houde *et al.* (7, 8) have studied the ability of graded doses of nalorphine to antagonize the analgesic activity of 5 and 10 mg of morphine observing a complete antagonism in a dose ratio morphine-nalorphine of 4:1, but in a ratio 2:1, the antagonism was only partial, and with the ratio 1:1 the analgesic effect was higher than that of the same dose of morphine alone.

These results encourage further studies in order to clarify the mechanism of the action of nalorphine at different ratio levels.

TABLE I

Effect of different concentration of nalorphine on the *in vitro* N-demethylation of morphine. The figures represent the amount of formaldehyde formed from morphine 1.3 × 10^{-3}M per gram of wet liver. (means ± S.E.M. of 6 experiments).

Time of incubation minutes	*Control* nmole*	*1.3 × 10^{-3}M*		*1.3 × 10^{-4}M*		*1.3 × 10^{-5}M*	
		*Δ nmole**	*P*	*Δ nmole**	*P*	*Δ nmole**	*P*
5	112 ± 7	− 41 ± 11	< 0.02	− 20 ± 9	< 0.10	+ 62 ± 11	< 0.005
10	191 ± 16	− 93 ± 30	< 0.05	− 34 ± 27	< 0.30	+ 45 ± 12	< 0.02
15	255 ± 21	− 122 ± 23	< 0.005	− 58 ± 20	< 0.05	+ 67 ± 10	< 0.005
60	527 ± 14	− 373 ± 23	< 0.001	− 80 ± 17	< 0.01	+ 95 ± 15	< 0.005
120	847 ± 29	− 603 ± 62	< 0.001	− 90 ± 32	< 0.05	+ 212 ± 29	< 0.001

*Δ are the differences obtained with morphine plus nalorphine, minus those with morphine alone in each experiment. P difference from zero calculated with Student's *t* test.

RESUMEN

Se estudia la influencia de diferentes concentraciones de nalorfina sobre la N-desmetilación *in vitro* de la morfina por homogenizados de hígado de rata. Se incuban sobrenadantes homogenizados de hígado (9.000 × g × 30 minutos) con morfina y nalorfina en relaciones de dosis 1:1, 1:0,1 y 1:0,01. La concentración de morfina fue en cada caso de $1{,}3 \times 10^{-3}$M. Cuando la relación de dosis morfina-nalorfina fue 1:1, este último alcaloide inhibió la N-desmetilación de la morfina en un 70% después de 120 minutos de incubación. En relación de dosis 1:0,1 la inhibición fue sólo de 10%. Por el contrario, cuando la relación de dosis fue 1:0,01, la nalorfina aumentó la N-desmetilación de la morfina en un 25%.

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