Failure of prolyl-leucyl-glycinamide to alter analgesia measured by the Takemori test in morphine-pretreated rats

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Van Ree & de Wied (1976) reported that subcutaneous (s.c.) microgram injections of prolyl-leucyl-glycinamide (PLG), the *N*-terminal side chain of oxytocin, facilitated the development of morphine tolerance, measured by the Takemori test. It was previously (Takemori et al 1973; Tulunay & Takemori 1974) noted that as little as one injection of morphine in mice caused an increased sensitivity to naloxone, indicating that the use of naloxone and morphine injections together would provide a very sensitive indicator of tolerance and physical dependence. Van Ree & de Wied found that 1 μg PLG, injected 1 h before a 20 mg kg^{-1} (morning) and again before a 40 mg kg^{-1} (afternoon) morphine HCl treatment injection, attenuated the hotplate analgesia produced the following morning by 80 mg kg^{-1} morphine given with 1 mg kg^{-1} naloxone. Using similar procedures we carried out several experiments which failed to confirm these results. Therefore, the following experiment using several morphine test doses, two PLG treatment doses, and two tests of analgesia, was carried out.

The experiment was conducted with 141 experimentally-naive, male Wistar rats (140–160 g), obtained two weeks earlier from Canadian Breeding Laboratories (Constant, Quebec). Maintenance was with free access to food and water under a 0800–2000 h light cycle. One day before the experiment the rats were transferred from group cages to single cages. The experiment, designed after that of van Ree & de Wied (1976), was two days long. On day 1, each rat received intraperitoneally (i.p.) 24 mg kg^{-1} morphine sulphate (BDH) at 1000 h and 48 mg kg^{-1} at 1700 h, each dose being dissolved in 0·9% NaCl (saline) (2 ml kg^{-1}). One h before these injections, each rat was injected s.c. with 0·2 ml saline containing 0, 1, or 10 μg PLG. The PLG (obtained from Organon, Oss, The Netherlands and Sigma Chemical) was dissolved in a drop of 0·001 M HCl and further diluted with saline. Commencing 0900 h the next day every rat received, at 0·5 h intervals, three hotplate tests, each followed 30 s later by one tailflick test. The hotplate procedure, after van Ree & de Wied (1976), used a stainless-steel hollow plate, maintained at 53 °C to produce 10–12 s response latencies in naive rats. The tailflick procedure was that described by Mucha et al (1978). To avoid tissue damage, both tests had maximum allowable latencies of 60 s. Immediately following the second tailflick test, each rat received one i.p. injection on each side of the abdomen. The first consisted of 12, 24, 48, or 96 mg kg^{-1} morphine, and the other 1 mg kg^{-1} naloxone hydrochloride (Endo), each dissolved in saline (2 ml kg^{-1}).

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For each rat the two predrug latencies of each test were averaged and the difference between the baseline and the third test was computed. A summary is presented in Table 1. Separate analyses of variance indicated highly significant overall effects of morphine for hotplate ($F(3,129) = 34{\cdot}2$, $P < 0{\cdot}001$) and tailflick tests ($F(3,129) = 54{\cdot}8$, $P < 0{\cdot}001$). However, there were no appreciable effects of the PLG: neither overall PLG ($F(2,129) = 0{\cdot}51$, $F(2,129) = 0{\cdot}11$ for hotplate and tailflick, respectively), nor PLG-by-morphine interactions ($F(6,129) = 0{\cdot}24$, $F(6,129) = 0{\cdot}35$) were significant.

These results, therefore, confirmed our initial failures to replicate the finding of van Ree & de Wied (1976) that s.c. administration of PLG before morphine pretreatments reduced the analgesia measured one day later in the Takemori test. Our negative findings cannot be accounted for by procedural variables, since the procedures used were designed to replicate those of van Ree & de Wied (1976). Moreover, we included a higher dose of PLG and a wider range of morphine test doses. Van Ree & de Wied (1976) noted that in pilot experiments PLG changed the log dose/response curve slope, which could result in a crossover of the curves for PLG and control groups. Since all our previous attempts to replicate their findings used only one morphine test dose, additional ones were required to ensure that we had not inadvertently chosen a dose falling at a crossover-point. In addition to this failure to find a PLG-related increase in the degree of morphine tolerance, we have also obtained negative or equivocal results of peptide treatment on morphine tolerance when the morphine was given without naloxone, and when the morphine and PLG (2 ng–20 μg) were given by intracerebroventricular injection.

Table 1. Mean (±s.e.)* increase (s) over baseline** hotplate and tailflick latencies of morphine-pretreated rats given 0, 1, or 10 μg PLG following various morphine doses combined with 1 mg kg^{-1} naloxone.

PLG dose (μg)	Test	Morphine dose (mg kg^{-1}) 12	24	48	96
0	Hotplate	3·4 ± 1·1	9·3 ± 2·9	19·1 ± 5·6	36·8 ± 5·7
	Tailflick	0·1 ± 0·4	7·9 ± 3·0	16·3 ± 6·9	44·1 ± 3·1
1	Hotplate	4·7 ± 2·2	13·9 ± 4·6	23·4 ± 6·0	37·9 ± 4·8
	Tailflick	2·8 ± 2·1	9·2 ± 5·3	12·2 ± 5·4	44·8 ± 5·1
10	Hotplate	3·8 ± 2·1	8·5 ± 1·0	17·8 ± 5·6	40·3 ± 5·2
	Tailflick	1·1 ± 0·4	6·1 ± 1·7	17·6 ± 6·1	40·0 ± 4·3

* Computed using data from 11–13 rats per dose.

** Average of two predrug measures taken in each rat; mean (±s.e.) hotplate baselines were 10·5 ± 0·4, 10·9 ± 0·3, and 10·5 ± 0·3 s for pooled 0, 1, and 10 μg PLG treatment groups, respectively; respective mean tailflick baselines were 2·8 ± 0·2, 3·4 ± 0·2, and 3·0 ± 0·2 s.

A number of groups reported that posterior pituitary peptides facilitate opiate tolerance and dependence (Cools et al 1977; de Wied & Gispen 1976; Krivoy et al 1974; van Ree & de Wied 1976; van Ree et al 1976). Recently, Walter et al (1978) reported an inhibition of morphine tolerance and physical dependence development in mice injected with a proposed competitive antagonist of PLG. In contrast, Schmidt et al (1978) found no effect of vasopressin or oxytocin on the level of tolerance attained after morphine pellet implantation for 3 days, or multiple i.p. injections for 5 days. One possible explanation of the difference in outcome is that the latter treatments produced maximal tolerance, and that the peptides increased the rate of acquisition but not the final degree of tolerance. This does not apply to the present results since they were obtained with the same methods as those used by van Ree & de Wied (1976). We have also found ambiguous effects of PLG and desglycinamide lysine vasopressin on the development of ethanol tolerance. In one study we found suggestions of impairment of tolerance by these peptides (Kalant et al 1978), but in a later unpublished replication we found no effect.

The subjects used by van Ree & de Wied (1976) were female rats while in the present study and in that of Schmidt et al (1978) they were male; therefore, the sex of subjects may prove to be a critical variable for understanding some peptide effects. However, in other experiments using male rats we found reproducible significant effects of neurohypophyseal peptides on ethanol consumption (Finkelberg et al 1978; Mucha & Kalant 1979). It seems probable that the effects of these peptides on acquisition of new behaviours are considerably more complex than has been so far recognized.

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Note in Proof

Recently, Walter et al (1979) reported that PLG prevented rather than facilitated development of pellet-induced physical dependence on morphine in mice.

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The potassium ion selective electrode as a tetraphenylborate sensor for quaternary ammonium salts analysis

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Recently, some solid-state or liquid membrane selective electrodes, including the commercial calcium electrode (Orion, model 92–20), that have a Nernstian-like response to cationic detergents have been described (Baiulescu & Cosofret 1977). An especially prepared perchlorate selective membrane electrode was described (Vosta & Havel 1973) for potentiometric titrations of cetylpyridinium salts with perchlorate anion, but, due to the limited linear response region of the electrode, a relatively high concentration of quaternary ammonium compound (0·0235 M) was necessary for satisfactory analyses.

We have reported on potentiometric determinations of several quaternary ammonium compounds using a preconditioned silver electrode (Pinzauti & La Porta 1979). In a continuation of the applicability of potentiometry in cationic surfactants analysis, a commercial organophilic porous membrane electrode for potassium ion (Orion, model 93–19) was tested as indicating electrode using a standard 0·01 M sodium tetraphenylborate solution as the titrant.

2 ml of 0·2 M acetate buffer (pH 3·4) and 0·4 ml of 6 M NaCl solution were added to 20 ml of 0·0005–0·001 M stirred solution of cationic surfactant. The titrant was delivered in equal increments (0·025 ml) from a piston microburette (Metrohm, E457). Potentials of the potassium electrode were referred to a Metrohm EA404 saturated calomel electrode assembled into a Beckman remote junction filled with a 2 M NaCl solution. Potentiometric measurements were performed with a Metrohm E500 digital pH-meter capable of detecting change in potential of 0·2 mV. A typical titration curve is shown in Fig. 1; the end point was taken as the volume

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