Deamination Assay. CR-CdR deaminase was prepared from two different sources: (1) human liver following the procedure of Wentworth and Wolfenden;[5] (2) AML blast cells,[4] by ammonium sulfate fractionation and DEAE column chromatography. The assay procedure has been published previously.[5] Under these conditions 50% of *ara*-C was deaminated in 45 min. Using the same assay conditions, no significant deamination of cytaramin or cytarazid was detected in 8 h.

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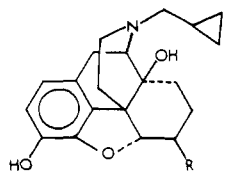
6β-[*N*,*N*-Bis(2-chloroethyl)amino]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (Chloranaltrexamine), a Potent Opioid Receptor Alkylating Agent with Ultralong Narcotic Antagonist Activity

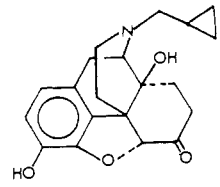
Sir:

Agents which selectively form covalent bonds with opioid receptors, both in vivo and in vitro, would find wide use as pharmacologic probes and might prove to be of considerable clinical value as ultralong-acting narcotic antagonists. For these reasons there have been a number of attempts to develop such drugs.[1-7] In this communication we report on the first example of such a compound, 6β-[*N*,*N*-bis(2-chloroethyl)amino]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (**1**), which we have named chlornaltrexamine (CNA).



1 (CNA), R = $N(CH_2CH_2Cl)_2$
3, R = $N(CH_2CH_2OH)_2$
4, R = NH_2

The design rationale involved modification of naltrexone (**2**),[8] a powerful narcotic antagonist, by attachment of a



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reactive moiety to C-6. This position was selected for modification because the introduction of groups at this location does not abolish antagonist activity.[9-11]

Addition of divided amounts of $NaCNBH_3$[12] over a period of 90 h to a mixture of **2**·HCl, diethanolamine, and molecular sieves in methanol[13] maintained at ambient temperature afforded **3** (44%),[14] which was purified by dry column chromatography (silica gel, 90:10:4 EtOAc–MeOH–NH_4OH) and was crystallized (MeOH–Et_2O) as **3**·2HCl: mp 205–207 °C; $[\alpha]_D$ −133.4° (*c* 0.5, MeOH). Anal. ($C_{24}H_{36}N_2O_5Cl_2$·0.5MeOH) C, H, N. An alternate synthesis of this intermediate involved the reaction of the 6β-amino compound **4**[11] with ethylene oxide.[15] The conversion of **3**·2HCl into **1**·2HCl (42%) was effected by a modification of the triphenylphosphine–CCl_4 procedure[16] using DMF as solvent. After the mixture was kept at 4 °C for 16 h, the residue (free of DMF) was purified by extraction (EtOAc) and dry column chromatography (silica gel, 98:2 Et_2O–NH_4OH). Pure **1**, EIMS *m/e* 468 (M^+), TLC R_f 0.63 (silica gel, 100:1 Et_2O–NH_4OH), was converted to the dihydrochloride salt: mp 185–195 °C; $[\alpha]_D$ −126° (*c* 0.5, MeOH). Anal. ($C_{24}H_{34}N_2O_3Cl_4$) C, H, N, Cl.

Using the tail-flick assay[17,18] CNA had no analgesic effect of its own at doses of 0.6, 1.2, 2.4, and 4.8 nmol/mouse 2 h after icv[19] injection. A dose of 4.8 nmol/mouse of CNA produced analgesia in 18% of the mice when tested 10 and 20 min after the injection. This analgesic effect was no longer apparent after 60 min. Saline controls had no effect. This same dose of CNA (4.8 nmol/mouse) also had a lethal effect in 12% of the animals injected which was manifested within 1.5 h of the injection. The only other dose with lethal effects was the 2.4 nmol/mouse dose which caused death in 2% of the animals.

Analgesia[17,18] was measured 30 min after sc morphine injection and 2 h after icv injection of either saline, naltrexone (2.4 nmol/mouse), or CNA (0.6, 1.2, or 2.4 nmol/mouse). In contrast to naltrexone which showed no residual inhibition of morphine-induced analgesia 2 h after icv injection, CNA exhibited significant dose-dependent inhibition. Doses of 0.6, 1.2, and 2.4 nmol/mouse of CNA increased the ED_{50} of morphine of 5.0 (3.5–7.2) mg/kg by 7-, 66-, and 179-fold,[20] respectively. In addition, the antagonistic property was apparent as long as 3 days after a single icv injection of the antagonist but could not be detected after 6 days (Figure 1). In preliminary studies, CNA was also an effective antagonist when administered parenterally and the effect lasted about the same duration as that after icv administration.

To show that CNA was inhibiting morphine analgesia by binding to the same receptor system, naloxone pretreatment was demonstrated to block the effect of icv injected CNA on morphine analgesia when it was tested 24 h after CNA administration. In control animals a threefold increase in morphine ED_{50} was observed 24 h after CNA treatment. Naloxone had no residual antagonistic effect at 24 h after administration. The dose required to block the effect of CNA (1.2 nmol/mouse) was 150 mg/kg divided into three injections given in a span

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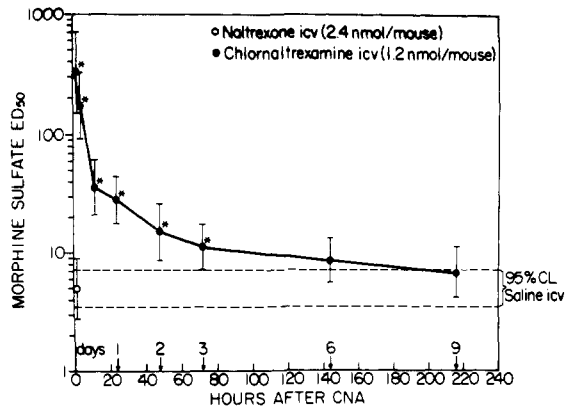

Figure 1. The duration of the inhibitory effect of chlornaltrexamine (CNA, 1) on morphine analgesia. The vertical bars represent 95% confidence intervals of the ED_{50} values. Asterisks indicate those ED_{50} values which are significantly different from the control value. The antagonistic effect of naltrexone was tested 2 h after administration and its effect had already dissipated.

of 30 min. The high dose of naloxone may be required because of the type of interaction which CNA may have with the opioid receptors. If CNA binds covalently to the receptors after one or only a few associations with the receptor, then essentially continuous occupation of the receptor by naloxone would be required to block the effect of CNA. Also, if CNA has an affinity constant similar to that of naltrexone, of which it is a derivative, then it would have a much higher affinity for the receptor than naloxone. This difference might also partially account for the requirement of a high dose of naloxone for blockade of the effect of CNA. This dose of naloxone also allows a long exposure of the receptor to naloxone which assures that it will be present at the receptor as long as the unbound CNA remains in the brain.

In vitro binding studies also are consistent with covalent association of CNA with opioid receptors. Inhibition of [^{3}H]naloxone to putative narcotic receptors was performed by a method similar to that of Pert and Snyder[21] as modified by Pasternak et al.[22] Brain homogenate preparations were preincubated for 5 min with a concentration of agonist or antagonist which initially inhibited about 80% of the [^{3}H]naloxone binding. Neither naltrexone- nor levorphanol-treated preparations exhibited any inhibition of [^{3}H]naloxone binding after four washes (centrifugation and resuspension of pellet in buffer) which was in contrast to the 40% inhibition of binding remaining in the CNA-treated preparation after this number of washes.[23] There were no significant differences between the preparations containing NaCl and no NaCl with regard to the loss of inhibition of [^{3}H]naloxone binding by the washing procedure.

These initial studies suggest that CNA produces ultralong narcotic antagonism through a covalent association with the same receptors which reversibly bind naloxone and naltrexone. This agent should be a useful probe for studying opioid receptors. Investigation of the role of the alkylating moiety in the structure–activity relationship of congeners of CNA is underway.

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