## Morphine Antagonistic Effect of Chlordiazepoxide (Librium®)

A previous paper from this laboratory[1] reported that chlordiazepoxide antagonizes the effect of morphine in mice in the tail flick test. The experiments leading to this observation were the consequence of a paper by CARRABATEAS and HARRIS[2] in which the narcotic antagonistic properties of a series of 1,4-benzodiazepines, derived from certain methadone-like analgetics by cyclization, were reported. A further study concerning the dose response relationship for chlordiazepoxide as a morphine antagonist is reported below.

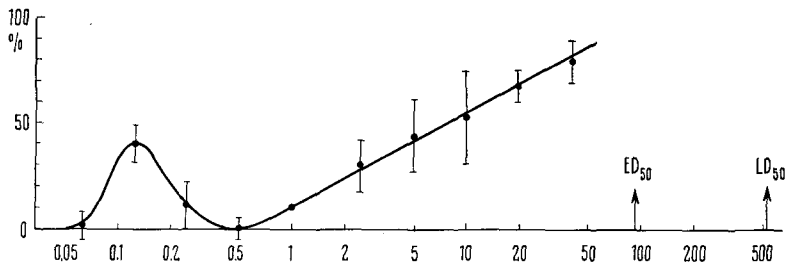
The D'AMOUR and SMITH tail flick test[3], modified for mice, was used. A thermic stimulus was obtained by means of a light bundle focused on the root of the tail of white, male mice of the NMRI strain, born and kept under conventional conditions. The reaction time of the animals was measured 30 min after a s.c. injection of morphine hydrochloride. The cut off point of irradiation time was selected to 15 sec, this being taken as 100% analgesia. The reaction time of a saline treated control group (about 5 sec) served as the 0% analgesia point. The drugs were dissolved in saline and administered in a volume of 10 ml/kg. Varying doses of chlordiazepoxide were given s.c. just prior to a fixed 5 mg/kg dose of morphine hydrochloride. Each test group consisted of 10 mice.

As the susceptibility of mice to morphine[4] and to chlordiazepoxide[5] is known to be subject to circadian changes, each dose of chlordiazepoxide was tested 4 or 5 times per day and the mean value of the results from these experiments recorded. Control groups treated with saline and morphine were also tested 4 or 5 times per day and the mean values of the results obtained in these tests (40–50% analgesia) were used for the calculation of the antagonistic effect of chlordiazepoxide.

The Figure shows the dose response curve for chlordiazepoxide. For each dose of chlordiazepoxide the mean ± S.D. of the daily mean values is indicated. Furthermore the s.c. $LD_{50}$ (530 mg/kg) and the s.c. $ED_{50}$ for muscle relaxant effect (94 mg/kg) reported by RANDALL et al.[6] are given. It will be seen that there are 2 phases of antagonistic activity, one with a maximum at 0.125 mg/kg and another beginning at 0.5 mg/kg and rising in a linear course.

The mechanism underlying the biphasic antagonism of chlordiazepoxide to morphine analgesia is obscure. The low dose antagonism may be due to a CNS alerting effect similar to that reported by BOISSIER et al.[7], who found chlordiazepoxide to potentiate the toxicity of D-amphetamine in aggregate mice when given s.c. in doses from 0.125–0.75 mg/kg. Higher doses (≧ 32 mg/kg) antagonized the toxic effects of D-amphetamine. Concerning the second phase of morphine antagonism the observations of MONNIER and GRABER[8] should be stressed. These authors found that i.v. injections of 30 and 40 mg/kg of chlordiazepoxide to rabbits produced an enhancement of the cortical response to reticular and hippocampal stimulation. This enhancement may lead to an increase in the reactivity of the animals to the pain producing stimulus. As the results reported by MONNIER and GRABER[8] only apply to doses of 30 and 40 mg/kg chlordiazepoxide, this hypothesis can, however, not be extended to the whole dose range.

On the other hand, it cannot be excluded that one and the same mechanism (e.g. competitive) is responsible for both phases of antagonism. No analgesic effect of chlordiazepoxide could be demonstrated with the mouse tail flick method. However GUPTA and GAITONDE[9] found potentiation of morphine in rats after 10 mg/kg i.p. of chlordiazepoxide. The intermediary phase of decreasing antagonism with a zero point at 0.5 mg/kg may be the expression of a similar morphine potentiation partially masked by CNS-stimulation and soon extinguished when the dose of chlordiazepoxide is further increased[10].




Dose response curve for chlordiazepoxide. Abscissa: mg/kg s.c. Ordinate: Inhibition in per cent of the morphine induced analgesia. $ED_{50}$ is the $ED_{50}$ for muscle relaxing effect. This value and the $LD_{50}$ are taken from RANDALL et al.[6]. The vertical bars represent the SD.

*Zusammenfassung.* Die morphinantagonistische Wirkung von Chlordiazepoxid (Librium®) wurde untersucht. Die Dosis-Wirkungs-Kurve zeigt 2 ansteigende Phasen mit einer intermediären, absinkenden Phase. Verschiedene Möglichkeiten zur Erklärung dieses Phänomens werden diskutiert.

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