ANTI-NOCICEPTIVE ACTIVITY OF CLONIXIN IN RHESUS MONKEYS

V.B. CIOFALO, J. PATEL and R.I. TABER

Department of Pharmacology, Biological Research, Schering Corporation, Bloomfield, New Jersey 07003, U.S.A.

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Clonixin (Sch 10304) [2-(2’ methyl-3’ chloro anilino) nicotinic acid] has been reported to be an orally effective analgesic and a non-steroidal anti-inflammatory agent in rodents (1). Clonixin has no dependence liability in monkeys (2) and its analgesic effect is not antagonized by naloxone in mice and rats (3). Clinical results with clonixin have shown degrees of analgesic effects not usually observed with non-narcotic agents. Single oral doses of clonixin ranging from 300–600 mg produced pain relief comparable to that seen with parenteral administration of morphine (4).

Procedures employing electric shock in monkeys have been used by other investigators for analgesic assessment (5–7). Thus far, only narcotic and narcotic antagonist analgesics have been shown to be effective in this procedure. This investigation describes the analgesic properties of clonixin on behavior maintained by nociceptive electric shock in Rhesus monkeys (Macaca mulatta) using a shock-escape procedure.

METHODS

Two chair-restrained male monkeys were trained to escape a constant current shock administered to an exposed segment of their tails by depressing a lever located directly in front of them. This shock was delivered in the absence of a conditioned stimulus. The constant current shock source supplied from 0 to 7 (mA) to variable physiological loads (i.e. against animal tissue impedance) (8). An additional design feature limited transients (i.e. current spikes) both at onset and termination of the shock. Using this shock source, currents delivered to variable physiological loads up to 12,000 ohms could be held within 1 % of the selected shock level. The measured impedance of the monkey was found to be in the range of 1,500 ohms. With this impedance the actual current delivered to the monkey’s tail was within 0.2% variation. Electrolytic paste was applied to the exposed segment of the tail before application of the electrodes. Standard behavioral equipment was used to control the experiment.

Each trial consisted of variable intervals ranging from 15–45 sec followed by a 10 sec shock. If the animal pressed the lever, the shock was terminated and this constituted a response. Shock was maintained at the same intensity for one block of 10 successive trials. The percent response was calculated as the number of positive responses/number of trials (10) at the designated shock intensity. Initially, low shock intensities were used, then
intensity was gradually increased in steps of 0.2 mA until a maximum response level (80–100%)
had been attained. Minimal intertrial responses were recorded at higher shock intensities. Subjects
were tested for a maximum of 15 blocks of 10 trials, which required approx., 3 hr, after which they were returned to individual home cages. A typical experiment required two days. On day 1, subjects were given pre-drug trials to establish a stable baseline. On day 2, subjects were tested after the administration of either vehicle or drug. Clonixin was administered orally by intubation using the free acid suspended in methylcellulose vehicle (0.5%; vol. 1 ml/kg), 1 hr prior to testing. Codeine phosphate served as a positive control, was dissolved in water and administered s.c. (vol. 1 ml/kg) 30 min prior to testing. Experiments were run at weekly intervals to minimize exposure, reduce behavioral fixation, and insure adequate drug elimination. An adequate time interval was allowed for normalization of behavior before each experiment began. Throughout the experiment white noise background was used to mask extraneous stimuli.

RESULTS AND DISCUSSION

The $S_{150}$ (that shock intensity at which the animal responded by pressing a lever, 50% of the time) and 95% fiducial limits were determined for each session. Activity ratios with 95% fiducial limits were used to compare the pre and post-drug shock-response curves. These comparisons were calculated according to the Maximum Likelihood Potency Probit Analysis Method (9).

| Treatment   | Dose (mg/kg) | Route | $S_{150}$ (95% fiducial limits) | Activity ratio (95% fiducial limits) | $p^2$ |
|-------------|--------------|-------|---------------------------------|-------------------------------------|-------|
| Pre-drug    | 3.85         | p.o.  | 4.01 (3.73–4.75)                | 1.00 (0.92–1.10)                    | N.S.  |
| Methylcellulose | 1 ml/kg     |       | 1.71 (1.29–2.15)                |                                     |       |
| Pre-drug    | 5.0          | s.c.  | 1.78 (1.33–2.33)                | 0.73 (0.39–1.10)                    | N.S.  |
| Pre-drug    | 9.0          | s.c.  | 3.91 (3.78–5.01)                |                                     |       |
| Codeine     | 10.0         | s.c.  | 4.76 (4.50–5.69)                | 1.22 (1.17–1.27)                   | <0.05 |
| Pre-drug    | 3.63         |       | 3.63 (3.45–3.81)                |                                     |       |
| Clonixin    | 12.5         | p.o.  | 3.89 (3.74–4.01)                | 1.07 (1.01–1.15)                   | <0.05 |
| Pre-drug    | 3.52         |       | 3.52 (3.24–3.99)                |                                     |       |
| Clonixin    | 25.0         | p.o.  | 4.16 (3.93–4.55)                | 1.16 (1.08–1.26)                   | <0.05 |
| Pre-drug    | 3.57         |       | 3.57 (3.44–3.72)                |                                     |       |
| Clonixin    | 50.0         | p.o.  | 4.74                           | 1.33 (1.28–1.39)                   | <0.05 |

1. $S_{150}$ is that shock intensity which produced a 50% response (reflected in escape from shock stimulus by pressing lever).
2. Statistical evaluation represents comparisons between pre-drug and drug treatment and calculated using the Maximum Likelihood Potency Probit Analysis Method (Finney, 1964).
3. Not significant.
4. Impossible to compute limits due to steepness of the shock-escape curve.
TABLE 2. Comparative shock escape thresholds after pre-drug and drug treatment in rhesus monkey No. 881.

| Treatment   | Dose (mg/kg) | Route | SI\textsubscript{50} (95% fiducial limits) | Activity ratio (95% fiducial limits) | P²  |
|-------------|--------------|-------|------------------------------------------|---------------------------------|-----|
| Pre-drug    |              |       | 3.11(2.86–3.43)                          | 1.06⁷                          | N.S.⁴|
| Methylcellulose | 1 ml/kg | p.o.  | 3.27(3.13–3.46)                          |                                 |     |
| Pre-drug    |              |       | 2.21(1.55–3.15)                          |                                 |     |
| Codeine     | 5.0          | s.c.  | 1.63(1.00–3.00)                          | 1.04(0.74–1.58)                 | N.S.|
| Pre-drug    |              |       | 2.46(2.25–2.67)                          |                                 |     |
| Codeine     | 10.0         | s.c.  | 4.20(4.00–4.44)                          | 1.71(1.56–1.90)                 | <0.05|
| Pre-drug    |              |       | 3.56(3.31–3.83)                          |                                 |     |
| Clonixin    | 12.5         | p.o.  | 3.98(3.73–4.61)                          | 1.21(1.07–1.40)                 | <0.05|
| Pre-drug    |              |       | 3.27(3.13–3.46)                          |                                 |     |
| Clonixin    | 25.0         | p.o.  | 4.37(4.15–4.58)                          | 1.34(1.26–1.41)                 | <0.05|
| Pre-drug    |              |       | 3.72(3.57–4.20)                          |                                 |     |
| Clonixin    | 50.0         | p.o.  | 4.92(4.74–5.15)                          | 1.34(1.26–1.43)                 | <0.05|

1. SI\textsubscript{50} that shock intensity which produced a 50% response (reflected in escape from shock stimulus by pressing lever).
2. Statistical evaluation represents comparisons between pre-drug and drug treatment and calculated using the Maximum Likelihood Potency Probit Analysis Method (Finney, 1964).
3. Activity ratio based on estimation since lines were not found to be parallel.
4. Not significant based on overlapping SI\textsubscript{50} and 95% fiducial limits.

Tables 1 and 2 show comparative shock threshold effects for each monkey before and after drug treatment.

In subject No. 7 both codeine, 10 mg/kg, and clonixin elevated the aversive shock threshold. A substantial and statistically significant (p<0.05, Table 1) effect was obtained with 10 mg/kg of codeine. The responses to clonixin were dose related. The lowest dose (12.5 mg/kg) produced a small but statistically significant (p<0.05) rise in shock level. The two higher doses (25 and 50 mg/kg) produced substantial and statistically significant (p<0.05, Table 1) increases.

In subject No. 881, codeine, 10 mg/kg, and clonixin also increased the aversive threshold. Substantial and statistically significant (p<0.05, Table 2) effects were obtained with 12.5 and 25 mg/kg of clonixin. These effects were greater than those observed in the first monkey with the same doses. In this monkey, clonixin, 50 mg/kg, showed an equivalent effect in elevation of aversive shock threshold in relationship to that observed in the first monkey.

Neither a lower dose of codeine (5 mg/kg) nor treatment with control vehicle produced any significant change in shock threshold. This is of special interest, for although the pre-drug control levels within any one subject varied from week to week, the comparison within a week (Day 1 vs Day 2) of any one experiment never showed a statistically significant difference with either vehicle control or a sub-effective dose of codeine.

Fig. 1 shows the results of a dose-response relationship obtained with clonixin for anti-nociceptive activity in Rhesus monkeys Nos. 7 and 881. The anti-nociceptive effect...
FIG. 1. Percent maximum response of clonixin on shock escape threshold in two rhesus monkeys.

(SI₅₀) obtained at each dose was expressed as the percentage of the Maximum Increase in Threshold Response (% MITR) according to the following formula:

\[
\text{Drug (SI₅₀) - Pre-drug (SI₅₀)} \times \frac{\text{Cut-off (mA) - Pre-drug (SI₅₀)}}{100} \times 100 = \% \text{MITR}
\]

This formula expresses the drug differences in threshold response relative to maximum response as determined by subtracting the pre-drug SI₅₀ from a pre-determined cut-off intensity (5.2 mA). The 5.2 mA cut-off was adopted to insure against any injurious effect to the tissue of the exposed tail.

When the results are expressed in terms of % maximum response, the dose-related nature of the clonixin effect becomes more evident (Fig. 1).

At the lowest dose tested, 12.5 mg/kg, the % MITR for monkeys Nos. 7 and 881 ranged from 19-29 respectively. The median dose, 25 mg/kg, reflected an increase in % MITR ranging from 43-64, and at the highest dose tested, 50 mg/kg, a peak ranging from 82-94% respectively was calculated.

It should be noted that no side effects were observed after any of the treatments in these monkeys. Also, this procedure has a built-in control for detecting neurological deficit in that animals grossly impaired would be unable to press the lever. Although both clonixin and codeine elevated the shock threshold, neither drug prevented the animals from responding at higher shock intensities.

As already noted, clinical results with clonixin have shown degrees of analgesia not usually observed with non-narcotic agents. Our results would tend to confirm the unique nature of the analgesic properties of this compound in that significant activity was observed at a dose similar to the clinical dose in a test procedure employing a stimulus and a species which has only been sensitive to narcotic and narcotic antagonist analgesics.
SUMMARY

Analgesic testing procedures with primates employing electric shock as a noxious stimulus are usually only sensitive to narcotic or narcotic antagonist analgesics. In this report, a non-narcotic anti-inflammatory analgesic, clonixin, has been shown to increase the level of shock tolerated by rhesus monkeys in a newly developed simplified measure of aversive shock thresholds. The effects produced by clonixin were dose-related over an oral dose range of 12.5-50 mg/kg. A s.c. dose of 10 mg/kg of codeine showed marked activity when used as a positive control.

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