Antipyrine – New Light on an Old Drug

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Abstract: The time courses of analgesic activity of 4 different tablets containing different amounts of antipyrine were determined in 14 volunteers using electrical tooth pulp stimulation to elicit pain. Drug action was monitored by following somatosensory evoked potentials obtained from electroencephalographic measurements as well as pain rating and pain threshold determination. The results were compared with data obtained after administration of 1000 mg acetaminophen and two different doses of aspirin (500 and 1000 mg). At the same time drug concentration in saliva of the same volunteers was analyzed by quantitative *in situ* thin-layer-chromatography to investigate the pharmacokinetics. Furthermore, the *in vitro* drug release from the different tablets was studied with a continuous flow cell model. Antipyrine produced reliable analgesic activity. The onset of action was significantly faster than after administration of the same dose of aspirin, and the effect lasted longer than after intake of the same dose of acetaminophen. Comparison of the drug action and drug level in the body showed an excellent correlation between pharmacodynamics and pharmacokinetics. The study confirms our earlier findings on the value of somatosensory evoked potentials as a method to investigate the pharmacodynamics of weak analgesics in humans. The results also suggest to reconsider the use of antipyrine as an over-the-counter analgesic.

Antipyrine (phenazone) is one of the oldest synthetic drugs and was introduced into medicine in the late nineteenth century. Very shortly after its introduction the drug was surpassed by an even more potent derivative, aminopyrine. Subsequently, because of the risk of agranulocytosis the use of aminopyrine was sharply curtailed, and though reports on agranulocytosis attributed to antipyrine have been rare, antipyrine has also lost favor (1). It has virtually disappeared as a therapeutic agent in the United States, but it is still available in over-the-counter-analgesics in some countries, e.g. in its birthplace Germany. In the U.S. today, the over-the-counter pain reliever market is dominated by two other old drugs, aspirin and acetaminophen.

The purpose of this study was to investigate biopharmaceutical, pharmacokinetic and pharmacodynamic aspects of antipyrine and compare its analgesic potency with that of aspirin and acetaminophen. Four different products containing antipyrine that are available as over the counter tablets in Germany were investigated. As a biopharmaceutical parameter the drug release from the tablets was studied with the use of a continuous flow cell model (2, 3). With this model it was possible to establish *in vitro*/*in vivo* correlations for aspirin tablets, which makes it possible to predict *in vivo* drug levels from *in vitro* dissolution data (4).

Drug concentrations were monitored by following the saliva levels of antipyrine. Saliva is more convenient to obtain than blood, and since antipyrine binds minimally to plasma protein, the plasma/saliva ratio is close to unity (5). Therefore, pharmacokinetic parameters obtained from saliva and plasma analysis are similar.

Finally, the pharmacodynamic activity was measured by following subjective pain rating and threshold determination as well as objective recording of somatosensory evoked potentials in human volunteers. This method has recently been reviewed (6) and has been applied by our group to study the time course and the relative potency of different weak analgesics and their combinations (7–9). Using this technique it was possible to correlate the pharmacokinetics of several analgesics obtained from saliva analysis with their pharmacodynamics (9).

The present study represents a first step to evaluate the pharmacological activity of antipyrine in humans using somatosensory evoked potentials. The effects of 1 g of aspirin and acetaminophen are compared with the same dose of antipyrine. Because in Germany antipyrine is mainly used in combination products and as this study was limited to commercially available over-the-counter drug products, three other investigated tablets contain also other analgesic ingredients. This limits the possibility of comparing different antipyrine doses, but allows the direct comparison of the pure drug products with these fixed combinations. No placebo was used, as only a relative comparison of different analgesics under controlled conditions was attempted.

Materials and Methods

The methods have previously been outlined in detail (2, 7, 8).

Subjects

Each drug was given to a group of 14 volunteers aged 21 to 36 years. 9 of the subjects were male, 5 female. Their weight range was 49 to 80 kg (mean 67 kg), their height 1.62 to 1.92 m (mean 1.77 m). The volunteers were students and research associates. The study took place in a specially designed separated quiet room. Subjects were informed about the experimental protocol and signed an informed consent form.

Tablet Preparations Used for the Study

The four tablet preparations studied contained antipyrine in different amounts: A (500 mg); B (200 mg); C (150 mg); D (130 mg). Other ingredients were 150 mg propyphenazone and 100 mg salacetamide (B), 150 mg phenacetin (C), 95 mg salicylate and 225 mg salicylamide (D). All of the four tablets contained 50 mg of caffeine. Aspirin and acetaminophen were used in tablets containing 500 mg of the analgesic.

Drug Application

Each drug preparation was evaluated in a double-blind, crossover design. A dose of two tablets was administered for each trial. The volunteers swallowed the tablets with their eyes closed with a total of 200 ml water and thoroughly rinsed the mouth to avoid interference of residual drug with the saliva assay. During the experiment the volunteers were not allowed

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to eat or drink. At least 48 h elapsed between two experiments on the same subject. Measurements for individual subjects were performed at the same time of day to avoid influences of diurnal rhythms.

Tooth Pulp Simulation and Saliva Level Monitoring

Bipolar electrical stimuli were applied to one of the upper incisors using conductive rubber discs mounted in an individual impression of the teeth. Each stimulated tooth was kept dry. The quality of the contact between the stimulating electrodes and the tooth was monitored. The electrical stimuli of 0.3 ms were applied stochastically with an interstimulus interval ranging from 1 to 3 s. The stimulus current was kept constant during the experiment. The reproducibility of the current used in the same individual on different trials were S_{rel} = 20.7 % (7), the range of current varied interindividually between 18.7 and 65 μA.

Five minutes before drug intake the threshold of sensation (lowest current necessary to evoke a sensation) was determined for each volunteer. This current was doubled and the resulting painful stimulus was applied and kept constant during the whole experiment. Measurements were performed immediately before drug intake (100 %) and at 20, 40, 60, 90, 120 and 150 min after drug intake. During the pain measurement saliva was collected simultaneously, the mouth was then emptied into glass vials. The saliva was frozen until analyzed. During the tooth stimulation an increase in saliva flow was observed.

Recording of the Evoked Potentials and Subjective Pain Response

Evoked potentials were taken from EEG recordings between C_z and F_8 (10–20 system). The signals were amplified and averaged using time constants of 0.3 s and an upper frequency limit of 70 Hz. For every evoked potential, 64 EEG samples were averaged. For the quantitative evaluation the height of the negative peak N 2 of the evoked potential (latency 170–260 ms) was measured (7), and its percentage of change after the drug intake was taken as one of the indicators for the pharmacodynamic activity. Pain ratings and the threshold of painful sensation were derived from verbal reports as described before (8) and calculated as the subjective percent change in the analgesic response.

Dissolution Method

The dissolution method used has been described in detail before (2, 3). In a water bath of 37°C continuous flow cells rotated for 360° forth and back with 1.2 cpm. The solvent used was gastric fluid USP XX, the flow rate 15 ml/min. Fractions were sampled at 3, 6, 9, 12, 18, 24, 30, 45 and 60 min and analyzed by HPTLC. Six tablets from 3 different lots of each of the four preparations were individually investigated. Before the dissolution experiment 15 tablets from the same lots were tested for antipyrine content. All tablets showed antipyrine content with less than ± 5 % deviation of the label.

Chromatography

The analytical method used for the dissolution experiments as well as for the saliva monitoring was quantitative high-performance thin-layer chromatography (3, 10). Antipyrine was measured by *in situ* remission at 255 nm. The quantitative analysis of the chromatograms was done by integration of the peaks and calibration curves of standard solutions chromatographed on the same plate.

Results and Discussion

Drug Release from the Tablets

Using a continuous flow cell dissolution model antipyrine was released from all of the investigated tablets within 12 minutes (Fig. 1). The dissolution rate was faster than the release of aspirin or acetaminophen from different tablets studied under the same conditions (2, 11). For aspirin and acetaminophen differences in the dosage form or the presence of other ingredients can cause significant changes in their dissolution behavior. In the case of aspirin it was possible to establish an *in vitro/in vivo* correlation which enabled one to predict the drug absorption rate with excellent precision (4). In the case of antipyrine this was not possible as the dissolution step is not the rate limiting step of absorption. Therefore even statistically significant differences of two tablets with fast drug release are of little meaning for predictions of *in vivo* situations. However, in all of the investigated tablets the pharmaceutical availability of antipyrine was fast and complete, so that bioavailability problems due to tablet formulation with this drug seem unlikely.

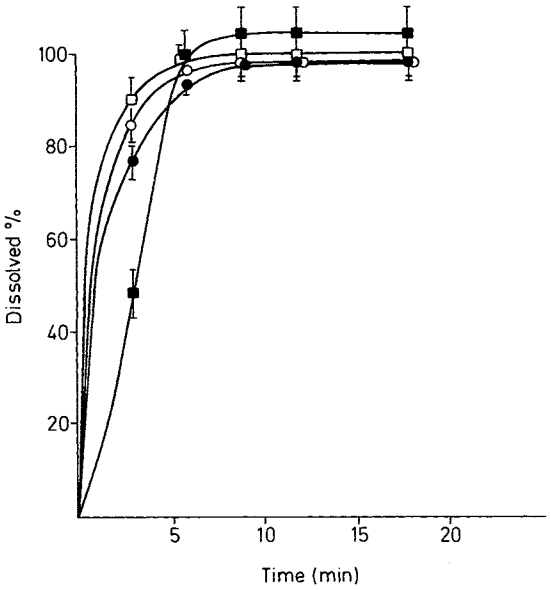

Fig. 1 In vitro dissolution profile of antipyrine determined for four different tablets with a continuous flow cell model (means of 6 tablets ± S.D.). Antipyrine content in the tablets was A (○, 500 mg), B (●, 200 mg), C (□, 150 mg), D (■, 130 mg).

Saliva Level Analysis

Pharmacokinetic studies on antipyrine using saliva as the biological reference fluid have resulted in the same kinetic parameters as obtained from plasma (5). The elimination half life is approximately 13 h, the apparent volume of distribution 64 l (5). We followed the saliva concentration of antipyrine during the absorption phase over 150 min (Fig. 2). Saliva levels and analgesic activity were measured in the same experiment. For tablet A containing only antipyrine as an analgesic active component a linear correlation (r = 0.96) between the saliva levels of antipyrine and the corresponding changes in the EEG was obtained. Similar linear correlations were also seen for aspirin and acetaminophen (9). Comparing the pharmacokine-

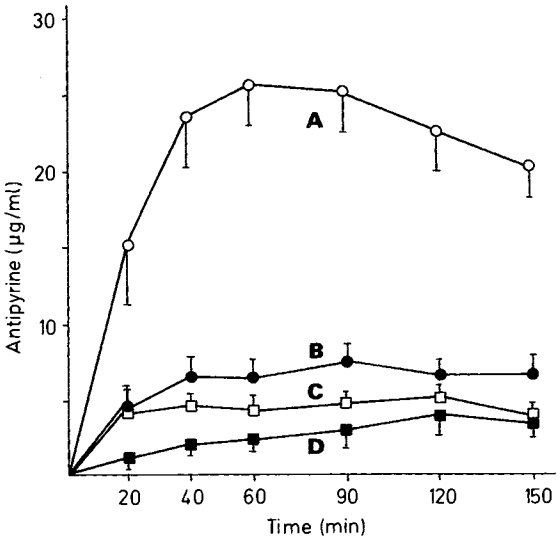

Fig. 2 Antipyrine concentrations in saliva after oral administration of 2 tablets of A (○), B (●), C (□), and D (■). Means of 14 subjects ± S.E.M.

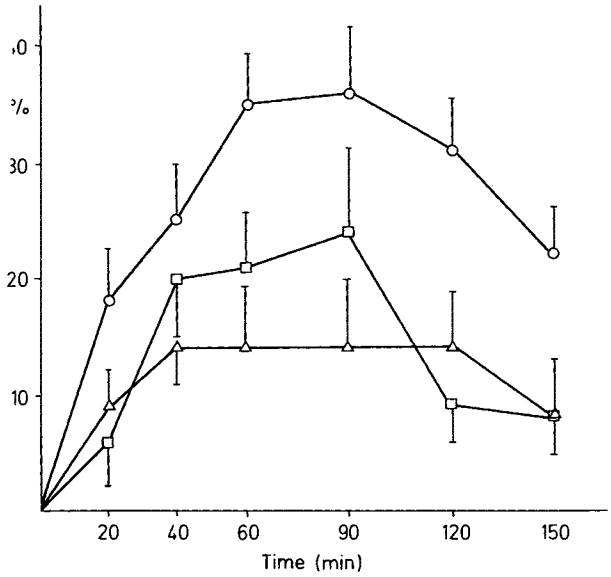

Fig. 3 Percent change (means of 14 subject ± S.E.M.) of the somatosensory evoked potential (○), the pain threshold (□) and the pain rating (△) following electrical tooth pulp stimulation and intake of 2 tablets of A containing 1000 mg antipyrine.

tic properties of antipyrine with those of aspirin and acetaminophen (12) there are some important differences. The elimination half life for a 1 g dose of antipyrine (~ 13 h) is significantly longer than for acetaminophen (2.5 h) and salicylate (5 h). Furthermore, the absorption step shows less variability for different antipyrine tablets than for aspirin products, since there is less influence of the dosage form on the absorption of antipyrine. In case of acetaminophen it is known that its rate of absorption depends on the rate of gastric emptying (13). We tried to standardize the gastrointestinal conditions by fasting the subjects before the experiment and giving all of the drugs with 200 ml water. Nevertheless, the average interindividual variation was significant higher for acetaminophen (S.E.M. 25 %) than for antipyrine (S.E.M. 12.8 %). Hence we can conclude that during the absorption phase the use of antipyrine will result in more reproducible drug levels in the body than the use of acetaminophen.

Measurement of Analgesic Activity

Tablet A contains only antipyrine as an analgesically active ingredient. The time/response curve for the three analgesiometric methods after administration of 1 g antipyrine is shown in Fig. 3. With all three methods maximum drug activity is seen between 60 and 90 minutes. The recording of somatosensory evoked potentials was the most sensitive method of the three showing changes up to 35 % of the signal measured before drug treatment. Threshold determination seems to be more sensitive than subjective pain rating.

In Fig. 4 the three parameters are compared for the tablets A, B, C and D. All four tablets contain the same amount of caffeine. However, previous investigations could not detect any difference in analgesic efficacy with the addition of caffeine to analgesic combinations (9). Therefore we may consider tablet A as a pure antipyrine-product. For the direct comparison of tablets A, B, C and D, this assumption is irrelevant as they all contain the same amounts of caffeine. The higher sensitivity in evoked potential recording results in a larger number of statistically significant differences between the investigated tablets. In general, tablet A shows the strongest effect, whereas tablet D is the weakest. The decrease of the evoked potential after administration of A (1 g antipyrine) was significantly more pronounced at any time point investigated than after administration of tablet D. Tablet B and C were not statistically different from each other at any time point investigated. Thus the tablet with the highest antipyrine content showed the strongest effect which was most readily detectable with the use of the evoked potential method for pain measurement. The fact that after evaluation of the evoked potentials more statistically significant differences between two different kinds of medication could be seen, which are only there as nonsignificant trends following pain-rating or threshold determination indicates the advantage of evoked potential monitoring in comparison to subjective methods. Even though pain is a subjective experience per se, it seems possible to monitor a neurophysiological correlate with higher sensitivity to measure drug activity. This agrees with previous results (7) that gave no significant difference between 500 mg and 1 g aspirin with the two subjective methods whereas the electroencephalographic method was able to differentiate significantly between the two doses.

Finally, the question was addressed as to how antipyrine compares to the two most common weak analgesics, aspirin and acetaminophen. Fig. 5 compares the effect of tablet A equivalent to 1 g antipyrine with the effect of 1 g acetaminophen and 500 mg and 1 g aspirin. From the results it is obvious that the onset of action is faster with antipyrine than with aspirin as seen by both subjective and objective methods. This may be due to the faster release of antipyrine from the tablet. The EEG method furthermore indicates a significantly stronger overall activity of 1 g antipyrine than 500 mg aspirin and a longer duration of action of antipyrine than acetaminophen. This fact may be due to the higher acetaminophen clearance.

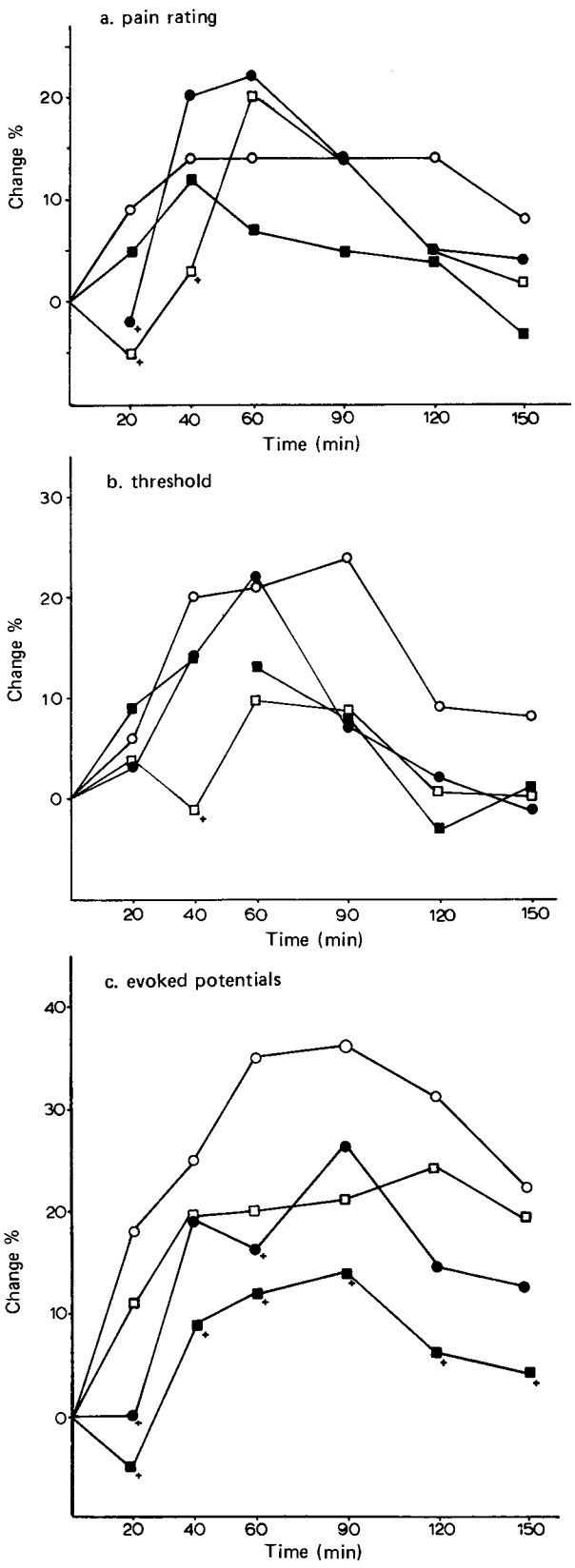

Fig. 4 Percent change (means of 14 subjects) of the pain rating (Fig. 4 a), the pain threshold (Fig. 4 b) and the somatosensory evoked potential (Fig. 4 c) following electrical tooth pulp stimulation and intake of 2 tablets of A (○), B (●), C (□), and D (■). Asterisks indicate significant differences ($p < 0.05$, Wilcoxon rank sum test) to tablet A.

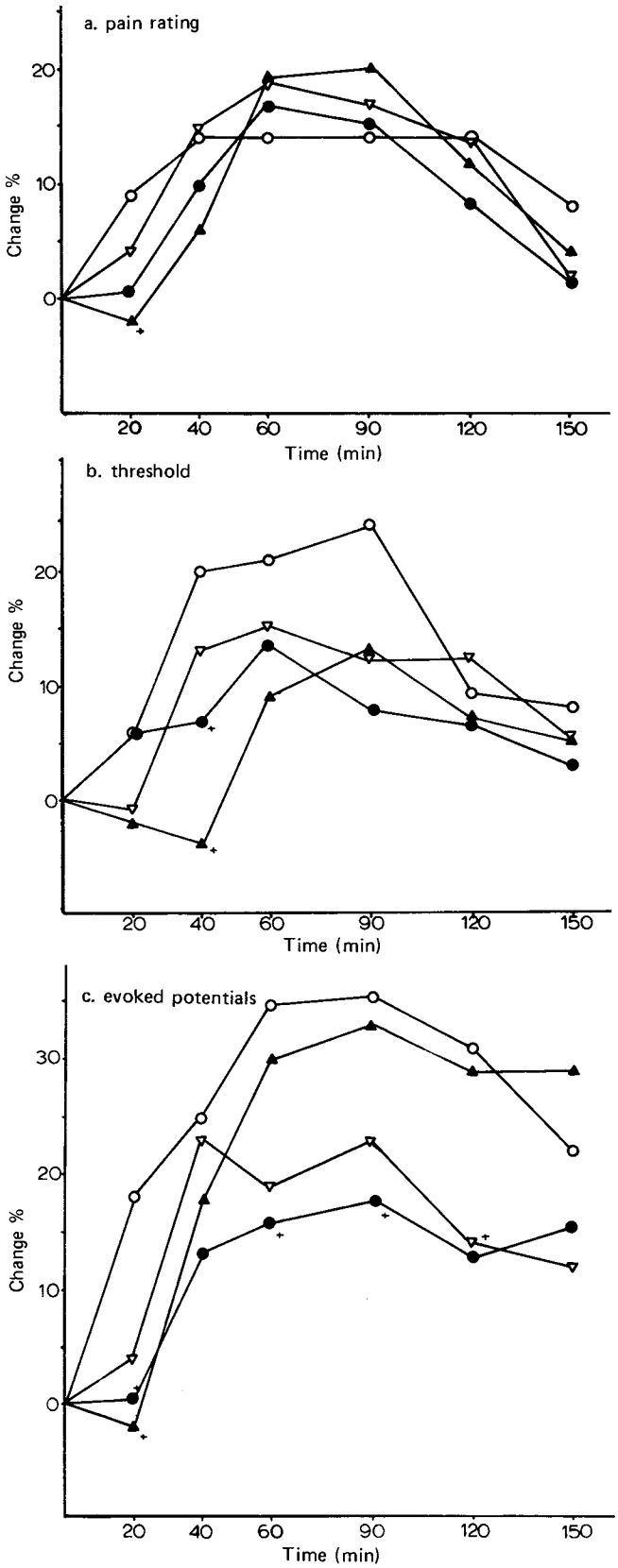

Fig. 5 Percent change (means of 14 subjects) of the pain rating (Fig. 5 a), the pain threshold (Fig. 5 b) and the somatosensory evoked potential (Fig. 5 c) following electrical tooth pulp stimulation and intake of 2 tablets A containing 1000 mg antipyrine (○), 1000 mg acetaminophen (▽), 500 mg aspirin (●) and 1000 mg aspirin (▲). Asterisks indicate significant differences ($p < 0.05$, Wilcoxon rank sum test) to antipyrine.

Conclusions

In the present study antipyrine has shown a fast drug release from tablets and rapid and reproducible absorption. Its analgesic efficacy is at least as good as that of aspirin and acetaminophen. Moreover, it seems that antipyrine shows an earlier onset of action than aspirin and a longer duration of action than acetaminophen. However, this comparison was only performed at a dose of 1 g of the three drugs. Although this dose is within the recommended dose range for acute pain treatment, more information is needed on the dose response curve for antipyrine as well as acetaminophen and aspirin.

As reports on serious side effects are rare, one should reconsider antipyrine as a useful alternative to the over-the-counter analgesics aspirin and acetaminophen. We propose additional clinical trials to obtain further information on the therapeutic value of antipyrine. The present study reconfirms the significance of evoked potential monitoring as a non-invasive tool to follow pharmacodyamics. The particular advantage of this method compared with traditional subjective methods is the fact, that a smaller number of subjects is needed to show differences between different kinds of drug treatment with statistical significance. In general, quantitative electroencephalography represents a valuable method in comparative drug efficacy studies by providing detailed information on the time course of action for drugs with effects on the nervous system.

References

(1) Woodbury, D. M., Fingl, E. (1975) in The Pharmacological Basis of Therapeutics (Goodman, L. S., Gilman, A., ed.), Macmillan Publ. Co. Inc., New York, 347–348.
(2) Derendorf, H., Rohdewald, P. (1981) Dtsch. Apoth. Ztg. 121, 493–498.
(3) Derendorf, H., Rohdewald, P. (1981) Dtsch. Apoth. Ztg. 121, 549–552.
(4) Derendorf, H., Drehsen, G., Rohdewald, P. (1983) Int. J. Pharm. 15, 167–175.
(5) Vesell, E. S., Passananti, G. T., Glenwright, P. A., Dvorchik, B. H. (1976) Clin. Pharmacol. Ther. 18, 259–272.
(6) Chudler, E. H., Dong, W. K. (1983) Pain 16, 221–244.
(7) Rohdewald, P., Derendorf, H., Elger, C. E., Knoll, O. (1980) Z. EEG – EMG 11, 199–204.
(8) Rohdewald, P., Derendorf, H., Drehsen, G., Elger, C. E., Knoll, O. (1982) Pain 12, 329–341.
(9) Derendorf, H., Drehsen, G., Rohdewald, P. (1982) Pharmacology 25, 227–236.
(10) Rohdewald, P., Drehsen, G. (1981) J. Chromatogr. 225, 427–432.
(11) Derendorf, H., Rohdewald, P. (1981) Dtsch. Apoth. Ztg. 121, 653–656.
(12) Brodie, B., Axelrod, J. (1950) J. Pharmacol. Exp. Ther. 98, 97–104.
(13) Forrest, J. A. H., Clements, J. A., Prescott, L. F. (1982) Clin. Pharmacokin. 7, 93–107.

REPORTS

Prostaglandin E_1-induced Catalepsy in the Rat: Role of Putative Neurotransmitters

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Abstract: Prostaglandin E_1 (PGE_1) produced dose-related catalepsy in rats when administered intracerebroventricularly. PGE_1-induced catalepsy was significantly inhibited after pretreatment with pharmacological agents known to attenuate central serotonergic and cholinergic activity. It was also inhibited by $PGF_{2\alpha}$ and naloxone. On the contrary, treatments enhancing central dopaminergic activity also reduced the cataleptic effect of PGE_1. The results suggest that PGE_1 induces catalepsy in rats by modulating activity of central neurotransmitters.

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Prostaglandins (PGs), particularly of the E series, have been reported to induce catalepsy, defined as inability to correct externally imposed postures, in several species including rats, both on peripheral (1) and central administration (2). PGE_1 is known to potentiate morphine (3) and cannabis induced catalepsies in rats, whereas $PGF_{2\alpha}$ and PG synthesis inhibitors attenuate the same (3, 4). Furthermore, PG synthesis inhibitors have been reported to reduce restraint stress induced potentiation of cannabis (5) and haloperidol (6) induced cataleptic responses in rats. This form of experimental stress is known to enhance rat brain PG activity (7). PGs are now thought to function as modulators of central monoaminergic (8, 9) and cholinergic (10) neurotransmission. The present study was undertaken to assess the role of central neurotransmitters in the cataleptic effect of centrally administered PGE_1.

Materials and Methods

Wistar strain albino rats (150–200 g), of either sex, were used. They were housed in individual perspex cages with free access to food (hind lever rat diet) and water, at ambient temperature of 22–25°C. Food was withdrawn 18 h prior to and water just before experimentation.

Catalepsy was initially assessed by a staging system (11). Quantification of catalepsy was done by the "ring test" (12), where the rat was placed on a steel ring, 12 cm in diameter, fixed to a steel stand at a height of 35 cm. The time during which the rat remained motion-