Psychopharmacology 59, 305–308 (1978)

Psychopharmacology

Naloxone-Reversible Analgesic Action of SKF 525-A in Mice

Thomas Lehman and George R. Peterson*

Departments of Pharmacology and Biological Chemistry, Wright State University School of Medicine, Dayton, Ohio 45435, U.S.A.

Abstract. SKF 525-A, given i.p. to mice at doses from 50 to 100 mg/kg, had analgesic activity approximately 40% the analgesic potency of *d*-propoxyphene HCl, a chemically similar narcotic. While the analgesia produced by propoxyphene was totally antagonized by naloxone, however, that produced by SKF 525-A was only partly reversed. We suspect that SKF 525-A may exert its antinociceptive actions partly by an interaction with CNS narcotic receptors and partly by a non-specific sedating action.

Key words: SKF 525-A – Analgesia – Propoxyphene – Naloxone – Narcotic receptors

SKF 525-A (2-diethylaminoethyl-2,2-diphenylvalerate), widely used as an inhibitor of the hepatic, microsomal system of mixed-function oxidases responsible for the biotransformation of most drugs (Conney, 1967), has long been regarded as being nearly devoid of pharmacological actions of its own (Cook et al., 1954). SKF 525-A thus finds wide use among workers in the field of drug metabolism to make distinctions between the role of a parent compound and that of its metabolites in the expression of pharmacological phenomena (Anders, 1971).

In a series of experiments concerning the toxicity of *d*-propoxyphene HCl (Darvon), we were able to account for certain toxic consequences on the basis of the SKF 525-A-like effects of propoxyphene on the hepatic metabolism of drugs (Hostetler et al., 1976, 1977; Peterson et al., 1977). We concluded that, given the chemical similarity between the two compounds, their common pharmacological actions are not surprising (Fig. 1).

We report further similarities between these two compounds, namely, analgesic action that may involve, at least in part, a direct interaction with central narcotic receptors (Snyder et al., 1974) or an indirect interaction involving release of endogenous opiate-like compounds (Hughes, 1975).

Goudie et al. (1975) observed that 50 and 100 mg/kg doses of SKF 525-A given i.p. to rats possessed sedative properties. Anders (1971) reviews other effects of SKF 525-A, such as its inhibition of monoamine oxidase, glucuronyl transferase, glycogen synthetase, and cholesterol biosynthesis. In addition to these peripheral actions, some central actions, in addition to those observed by Goudie et al. (1975), have been noted. For example, respiratory and gastrointestinal disturbances (Gaitonde and Borison, 1966), inhibition of muscle contractions (Harris and Milton, 1961), and activation of the pituitary (Magus et al., 1968) have been at least partly attributed to consequences of central effects of SKF 525-A.

Materials and Methods

Adult, male mice, weighing 22 to 27 g, of the NIH/Swiss strain (Laboratory Supply, Indianapolis, Indiana), were used. They were housed at 25°C under an automatic cycle (12 h light, 12 h dark).

The tail-flick method of D'Amour and Smith (1941) was used to assess the analgesic response to propoxyphene and SKF 525-A. The nociceptive response of untreated mice was determined, and a baseline response time of 2.0 s (SD = 0.4 s; $N = 10$) was established. The increase in latency in seconds above baseline was then used as a graded drug response. Since the thermal source that elicited the tail-flick automatically shut off at 8 s, the maximum increase in latency was 6.0 s.

To determine the ED_{50} and the 95% confidence limits for the analgesic drugs, the method of Litchfield and Wilcoxon (1949) was used. In this procedure, a probit transformation of a quantal response – the percentage of animals manifesting analgesia after drug administration – is plotted against the logarithm of the dose administered on log-probit paper. We arbitrarily designated any increase in latency more than 5 standard deviations (i.e., a 2 s increase in latency) above baseline as a positive for analgesia. Conversely, animals

* To whom offprint requests should be sent

0033-3158/78/0059/0305/$ 01.00

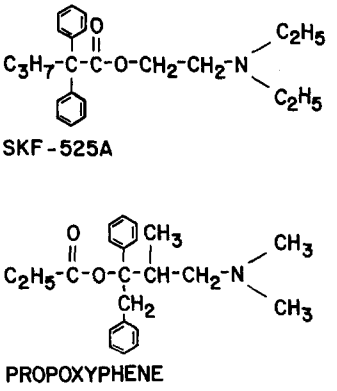

Fig. 1. Structure of SKF 525-A and propoxyphene

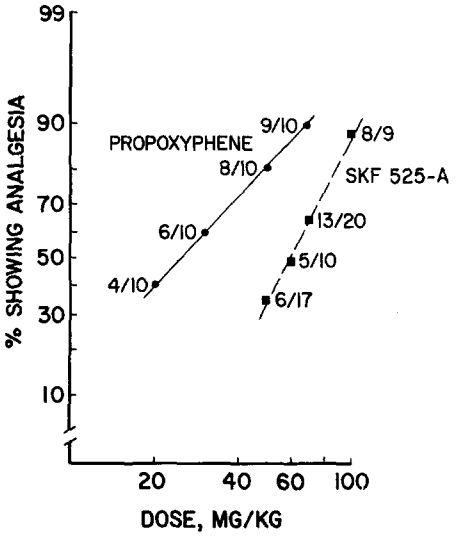

Fig. 2. Graded doses of propoxyphene HCl or SKF 525-A HCl were given i.p., and, 20 min later, the injected groups of mice were tested for analgesia using the tail-flick method. Numbers in parentheses indicate the number of mice scored as positives for analgesia over the number tested at that given dose

manifesting analgesia were scored as positives for reversal of analgesia if, 15 min after administration of naloxone, the increase in latency over baseline returned to less than 2 s.

The drugs, *d*-propoxyphene HCl, SKF 525-A HCl, and naloxone HCl were dissolved in distilled water and administered i.p. using a dosage volume of 0.1 ml/10 g body weight.

Student's *t*-test was used to distinguish differences between treatment groups. In cases of differences between groups where analgesia was considered a quantal phenomenon, the same test was used, the positives being scored 1 and the negatives 0.

Results

Figure 2 shows the results of the administration of graded doses of both propoxyphene and SKF 525-A administered i.p. to mice. The ED_{50} and the 95% confidence limits for propoxyphene was 24 (16–37 mg/kg), and for SKF 525-A it was 60 (51–71 mg/kg). SKF 525-A is thus approximately 40% as potent an analgesic as the narcotic propoxyphene.

There were other marked differences between the two drugs with regard to the character of the analgesia produced in the mice. For example, propoxyphene produced a syndrome of behavioral events (Straub reaction) characterized by muscular contractions that result in an erect tail, a hunched posture, extension of the hindlimbs, a prominent perineum, and increased locomotor activity. All narcotics over a wide dosage range will elicit this response in mice (Bilbey et al., 1960). SKF 525-A, on the other hand, produced a marked diminution of motor activity. At doses above 50 mg/kg, the animals were quite sedated, an observation reported by Goudie et al. (1975) for rats. The behavioral phenomena associated with narcotic administration, like the erect (Straub) tail, were not observed in the mice in the dosage range of 50–100 mg/kg of SKF 525-A.

The question of the specificity of the analgesic action of SKF 525-A was addressed. Analgesia and other narcotic-induced pharmacological actions are thought to be mediated by receptors in the nervous system possessing a high affinity for narcotics. The receptor-mediated actions of opiates are blocked or reversed by narcotic antagonists like naloxone, which tend to have even higher affinities for the opiate receptors than agonists (Snyder et al., 1974). A naloxone HCl dose of 4 mg/kg, which blocked or reversed the analgesia brought on by a high (20 mg/kg) dose of morphine sulfate, reversed the analgesia in all 18 of the mice given a dose of propoxyphene HCl (70 mg/kg) that produced analgesia in 90% (ED_{90}) of the animals. This dose of propoxyphene was chosen because higher doses would occasionally result in toxicity characterized by depressed locomotor activity, convulsions, and death. The analgesia caused by the equivalent (ED_{90}) dose of SKF 525-A HCl (100 mg/kg) was not always reversible by naloxone. Of 24 animals rendered analgesic by 100 mg/kg of SKF 525-A, only 16 manifested reversal after naloxone. The marked behavioral depression observed as a result of SKF 525-A administration was unaltered by the injection of naloxone; the depressed motor activity that ensued as a result of high doses of propoxyphene could be reversed by naloxone.

Different degrees of analgesia also are produced by the administration of equivalent doses of the two drugs. The mean latency period above baseline as a result of the injection of the ED_{50} dose of propoxyphene (70 mg/kg) was 5.8 ± 0.2 s. The latency period following the ED_{90} dose of SKF 525-A (100 mg/kg) was significantly shorter, 4.9 ± 0.2 s.

In addition to reversal by naloxone of the analgesic effect, blockade by naloxone of the antinociceptive action of SKF 525-A was also observed. After 4 mg/kg of naloxone HCl was administered i.p., SKF 525-A, injected 15 min later, was without effect on the tail flick latency in 9 of the 11 animals tested.

Discussion

The analgesic (and sedative) effects of SKF 525-A observed in these experiments must be considered in studies that utilize SKF 525-A as a pharmacologic tool in the evaluation of the role of microsomal metabolism in the manifestation of the effects of psychoactive drugs. This is especially necessary in studies that involve drugs with analgesic or CNS depressant actions.

Cook et al. (1954) observed that diphenylpropylacetic acid, a product of the metabolic hydrolysis of SKF 525-A, had marked CNS depressant effects of its own. It is interesting to note that Cooper et al. (1954) demonstrated that diphenylpropylacetic acid was as effective an inhibitor of cytochrome P-450-mediated biotransformations as the parent compound, SKF 525-A. Thus, the same part of the SKF 525-A molecule seems to be the key to both its sedative and microsomal inhibitory actions. Given the fact that a number of narcotics (methadone and congeners), such as methadone, propoxyphene, diphenoxylate, and *l*-α-acetylmethadol, have in common with SKF 525-A a diphenylmethane configuration (or a small variation of it), this same part of the molecule also might be responsible for the narcoticlike, naloxone-reversible analgesia observed for SKF 525-A.

The nature of the analgesia noted as the result of administration of SKF 525-A seems qualitatively different from narcotic (propoxyphene) induced analgesia. SKF 525-A, in addition to being a less potent analgesic, brings about a shorter latency of antinociceptive response than a comparable dose of propoxyphene. This observation could indicate a weak interaction of SKF 525-A with the opiate receptors or with endogenous opiates that mediate narcotic actions in the body. The fact that naloxone reverses or blocks SKF 525-A-induced analgesia constitutes our evidence that the analgesic action of SKF 525-A partly involves an interaction with these receptor sites in the nervous system.

There is some question, however, as to whether blockade or reversal of a drug's actions by naloxone can be taken as conclusive evidence that the drug in question is acting via an interaction with narcotic receptors. Although naloxone is often referred to in the literature as a 'pure' narcotic antagonist, it does have a number of other effects. Naloxone influences serotonin activity (higher levels of hydroxyindoleacetic acid in the CSF of man; Terenius et al., 1977). Holtzmann (1974) described inhibition of *d*-amphetamine-induced increases in locomotor activity in rats by naloxone. Grevert and Goldstein (1977) observed that naloxone increased the time that elapsed before untrained mice entered the dark box, perhaps by interfering with exploratory behavior, and Berkowitz et al. (1976) showed that naloxone would antagonize the analgesia produced by nitrous oxide. Thus, while SKF 525-A may interact directly with CNS narcotic receptors or indirectly by causing release of an endogenous opiatelike compound that then interacts with these receptors, it is conceivable that the analgesia produced by SKF 525-A is reversed by some heretofore unappreciated property of naloxone.

The inability of naloxone to reverse totally the analgesic actions of SKF 525-A seems to indicate that the pain-abolishing character of SKF 525-A is a consequence of factors other than those involving opiate receptors. The sedating, muscle-relaxing properties (Goudie et al., 1975; Harris and Milton, 1961) may account for some of the positive response of mice tested for analgesia on the tail-flick apparatus. High doses of sedatives can bring about analgesia (Harvey, 1975). The fact that the CNS depressant effect of the drug is not antagonized by naloxone is consistent with the notion that SKF 525-A's analgesic activity is a function of its sedating capacity as well as its possible interaction with narcotic receptors.

Acknowledgments. This work was supported by the Biomedical Research Support Grant Program, NIH Grant No. 1-S07-RR07155-01. We thank Eli Lily Company, Indianapolis, Indiana, for supplying *d*-propoxyphene HCl; Smith, Kline and French, Inc., Philadelphia, Pennsylvania, for SKF 525-A HCl; and Endo Laboratories, Garden City, New York, for naloxone HCl.

References

Anders, M. W.: Enhancement and inhibition of drug metabolism. Annu. Rev. Pharmacol. **11**, 37–56 (1971)

Berkowitz, B. A., Ngai, S. H., Finck, A. D.: Nitrous oxide "analgesia": resemblance to opiate action. Science **194**, 967–968 (1976)

Bilbey, D. L. J., Salem, H., Grossman, M. H.: The anatomic basis of the Straub phenomenon. Br. J. Pharmacol. **15**, 540–543 (1960)

Conney, A. H.: Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. **19**, 317–366 (1967)

Cook, L., Toner, J. J., Fellows, E. J.: The effect of β-diethylaminoethyl diphenylpropylacetate hydrochloride (SKF No. 525-A) on hexobarbital. J. Pharmacol. Exp. Ther. **111**, 131–141 (1954)

Cooper, J. R., Axelrod, J., Brodie, B. B.: Inhibitory effects of β-diethylaminoethyl diphenylpropylacetate on a variety of drug metabolic pathways *in vitro*. J. Pharmacol. Exp. Ther. **112**, 55–63 (1954)

D'Amour, F. F., Smith, D. L.: A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. **72**, 74–79 (1941)

Gaitondé, B. B., Borison, H. L.: Toxicity of beta-diethylaminoethyl diphenylpropylacetate in cats. Toxicol. Appl. Pharmacol. **8**, 118–125 (1966)

Goudie, A. J., Kelley, M., Taylor, M., Wheeler, T. J.: Acute sedative properties of SKF 525-A in rats: implications for its use as a metabolism inhibitor in the study of psychoactive drugs. Psychopharmacologia (Berl.) **41**, 291–294 (1975)

Grevert, P., Goldstein, A.: Some effects of naloxone on behavior in the mouse. Psychopharmacology **53**, 111–113 (1977)

Harris, E. D., Milton, A. S.: Inhibitory actions of SKF 525-A on muscle contraction. Proc. Soc. Exp. Biol. Med. **107**, 157–160 (1961)

Harvey, S. C.: Hypnotic and sedatives. In: Pharmacological basis of therapeutics (L. S. Goodman and A. Gilman, eds.), pp. 102–123. New York: Macmillan 1975

Holtzman, S. G.: Behavioral effects of separate and combined administration of naloxone and d-amphetamine. J. Pharmacol. Exp. Ther. **189**, 51–60 (1974)

Hostetler, R., Covault, H. P., Peterson, G. R.: Interactions between propoxyphene and mixed function oxidases in narcotic dependent mice. Fed. Proc. **36**, 991 [Abs.] (1977)

Hostetler, R., Reinhard, J. F., Peterson, G. R.: Model to study long term effects of propoxyphene on narcotic dependent mice. Res. Commun. Chem. Pathol. Pharmacol. **15**, 75–89 (1976)

Hughes, J.: Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. Brain Res. **88**, 295–308 (1975)

Lichtfield, J., Wilcoxon, F.: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. **96**, 99–113 (1949)

Magus, R. D., Rickert, D. E., Fouts, J. R.: Activation of hydrocortisone-induced tryptophan pyrrolase of rat liver by SKF 525-A: the possible involvement of antidiuretic hormone. Biochem. Pharmacol. **17**, 2071–2080 (1968)

Peterson, G. R., Hostetler, R., Covault, H. P.: Acute inhibition of microsomal metabolism by propoxyphene. Pharmacologist **19**, 211 [Abs.] (1977)

Snyder, S. H., Pert, C. B., Pasternak, G. W.: The opiate receptors. Ann. Intern. Med. **81**, 534–540 (1974)

Terenius, L., Wahlström, A., Agren, H.: Naloxone treatment in depression: clinical observation and effects on CSF endorphins and monoamine metabolites. Psychopharmacology **54**, 31–33 (1977)

Received December 7, 1977; Final Version March 17, 1978