The salivary results correlate closely with urinary data reported by Wall et al (1978), when V was indicated as not being a true sustained release product.

Because of the unsatisfactory dissolution profile for VI, saliva concentrations were measured after a volunteer had taken a 250 mg dose. This gave a total urinary recovery of 52·1%, a peak urinary excretion rate and a peak salivary concentration only one-third those of conventional tablets with a marked delay. The dissolution rate thus reflected the in vivo situation with all the products tested.

Our results contrast with the conclusions of Coppen et al (1969), Shaw et al (1974), and Bennie et al (1977) who found peak values for V occurring later than we did. Jeppsson & Sjogren (1975) have shown that the presence of food can delay lithium absorption. Recently, Johnson et al (1979) showed a statistically significantly delayed peak for V over a conventional product in fasted subjects, however, they too do not consider this product to be a true sustained release preparation. Tyrer et al (1976), as we did, administered tablets on an empty stomach and found the rates of absorption of V and I to be almost identical and release from V too fast to prevent potentially toxic blood concentrations of drug. It is clear that the administration of the tablet in relation to food may significantly affect the rate of lithium absorption, but overall there appears to be no real therapeutic advantage in using V as a sustained action product over conventional tablet formulations.

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# The influence of indomethacin on the acute toxicity of some anticholinesterases in mice

LEPOSAVA GRBOVIĆ, B. Ž. RADMANOVIĆ, *Department of Pharmacology, Medical Faculty, Belgrade, 1100, P.O. Box 662, Yugoslavia*

Recently we have reported that prostaglandins $E_2$ and $F_{2\alpha}$ ($PGE_2$, $PGF_{2\alpha}$) enhance the toxicity of carbachol, physostigmine and pilocarpine (Radmanović & Grbović 1979; Radmanović 1980). We have also found that relatively small doses of $PGE_2$ and $PGF_{2\alpha}$, injected intracerebroventricularly 15 min before cholinomimetic drugs, potentiated their stimulant action on the central nervous system. In the same doses, which exceeded several-fold the physiological concentrations of PGs in the brain, $PGE_2$ and $PGF_{2\alpha}$ inhibited acetylcholinesterase activity in various regions of the brain in cats both after in vivo i.c.v. injection and in vitro experiments (Grbović & Radmanović 1981). It is therefore possible that the potentiation of cholinomimetic drugs by $PGE_2$ and $PGF_{2\alpha}$ occurs, at least partly, as a result of the accumulation of acetylcholine in the central nervous system. In view of presented data we have investigated the influence of indomethacin, an inhibitor of prostaglandin biosynthesis, on the toxicity of some anticholinesterases.

Acute toxicities of physostigmine salicylate, neostigmine (Prostigmine Roche), Dyflos (DFP) and paraoxon given alone and when combined with drug treatment were determined in albino mice of either sex, 20–24 g. Drugs were dissolved in 0·9% NaCl and injected subcutaneously, with the exception of atropine sulphate which was injected intraperitoneally, in a volume of 0·1 ml per 10 g body weight. LD50 values, based on 24 h mortalities, were calculated by the method of Litchfield & Wilcoxon (1949); each group of animals consisted of 18 to 24 mice. In pilot experiments it was found that the used dose of indomethacin (10 mg $kg^{-1}$ s.c.) did not produce toxic signs by itself and that

* Correspondence.

Table 1. Effect in mice of indomethacin pretreatment (10 mg $kg^{-1}$ sc) on sc toxicity of anticholinesterases when given alone and when combined with atropine (10 mg $kg^{-1}$ ip).

| | LD50 mg $kg^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| Anticholinesterase | Control | Indomethacin pretreatment | *P* | With atropine | With atropine + indomethacin pretreatment | *P* |
| Physostigmine | 0·8 (0·69–0·93) | 1·32 (1·26–1·50) | <0·05 | 12·15 (9·3–15·7) | 18·2 (15·9–21·6) | <0·05 |
| Neostigmine | 0·28 (0·19–0·42) | 0·43 (0·34–0·63) | <0·05 | 1·3 (1·0–1·7) | 1·8 (1·3–2·2) | <0·05 |
| Dyflos (DFP) | 3·0 (2·7–3·3) | 3·53 (3·2–3·8) | <0·05 | 7·6 (6·0–9·3) | 8·5 (7·0–10·6) | >0·05 |
| Paraoxon | 0·27 (0·18–0·43) | 0·29 (0·23–0·41) | >0·05 | 0·76 (0·63–0·91) | 0·81 (0·73–0·95) | >0·05 |

95% confidence limits in brackets.

survival rate after 24 h was 100%.

The LD50 values obtained are given in Table 1. The toxic signs observed before death were typical of anticholinesterase poisoning: muscle fasciculations, lacrymation, salivation, general tremor, cyanosis, dyspnoea and convulsions.

In order to study the influence of indomethacin on the acute toxicity of cholinergic drugs, this inhibitor of porstaglandin biosynthesis in a dose of 10 mg $kg^{-1}$, was administered subcutaneously 30 min before the anticholinesterases. The results of these experiments showed that indomethacin decreased the toxicity of physostigmine, neostigmine and DFP and significantly increased the values of LD50 of these substances. The LD50 of physostigmine, neostigmine and DFP was enhanced by 39, 34 and 15%, respectively. Indomethacin also increased the values of LD50 of paraoxon, but the difference was not significant.

In order to study the influence of indomethacin on the protective effect of atropine (10 mg $kg^{-1}$, i.p.) in poisoning by anticholinesterases, indomethacin (10 mg $kg^{-1}$ s.c.) was injected into mice 30 min before atropine and anticholinesterases. Pretreatment with indomethacin enhanced the protective effect of atropine in physostigmine and neostigmine poisoning. After indomethacin the values of LD50 of physostigmine and neostigmine in mice protected by atropine, were enhanced by 32% and 26%. In experiments with dyflos and paraoxon the difference was not significant.

Our results showing that indomethacin decreased the toxicity of some anticholinesterases, are in agreement with our previous findings that prostaglandins $E_2$ and $F_{2\alpha}$ increased the toxicity of cholinergic substances (Grbović & Radmanović 1979; Radmanović 1980). Several studies have demonstrated that prostaglandins evoke contraction of the longitudinal smooth muscle of the intestine. Moreover, these compounds in low doses may potentiate the contraction elicited by acetylcholine, and by other smooth muscle stimulants (Harry 1968; Bennett et al 1968; Vane 1972; Grbović & Radmanović 1978). Gustaffson (1975) has shown that prostaglandins $E_1$, $E_2$ and $F_{2\alpha}$ in a dose-dependent manner enhance the contractile response of bovine iris sphincter muscle to transmural stimulation and to exogenous acetylcholine. Therefore, the potentiating effect of prostaglandins can be regarded as an interaction with cholinergic neuroeffector transmission. We reported that small amounts of $PGE_2$ and $PGF_{2\alpha}$ potentiated the stimulant action on the central nervous system of carbachol, pilocarpine and physostigmine. We have also found that these PGs significantly reduced the acetylcholinesterase activity in the caudate nucleus, thalamus and hypothalamus of the cat. Considering the fact that carbachol and pilocarpine are not substrates for acetylcholinesterase, any potentiation of their effects by anticholinesterase activity of PGs is likely therefore to be due to presynaptic actions of the cholinomimetic releasing acetylcholine. It is possible to assume that in experiments with physostigmine and PGs the inhibition of cholinesterase and the consecutive accumulation of endogenous acetylcholine are at least partly responsible for the resulting potentiation. This assumption is also supported by Poddibuik & Kleinrok (1976). These authors have found that prostaglandins E and F series increase the quantity of the total acetylcholine in the rat brain. Hanh & Patil (1972, 1974) suggested that increased salivation produced by prostaglandin $F_{2\alpha}$ occurs via the release of the endogenous acetylcholine.

Considering the fact that in our experiments indomethacin protects against anticholinesterase poisoning, the degree of protection is much less than that obtained by the antimuscarinic drug atropine. It suggests a minor role of prostaglandins in contributing to anticholinesterase toxicity by modification of cholinergic mechanisms. Also, neostigmine is a quaternary drug which does not readily pass the blood brain barrier and the results obtained with this anticholinesterase suggest that the minor role of prostaglandins in contributing to anticholinesterase toxicity appears to be important peripherally and centrally.

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# Plasma concentrations of trazodone and 1-(3-chlorophenyl)piperazine in man after a single oral dose of trazodone

S. Caccia*, M. H. Fong, S. Garattini, M. G. Zanini, *Instituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea, 62–20157 Milan, Italy*

Trazodone, a non-tricyclic antidepressant agent (for a review see Brogden et al 1981) is metabolized to form, among other metabolites (Yamato et al 1974, 1976; Baiocchi et al 1974; Jauch et al 1976) 1(3-chlorophenyl)piperazine (CPP) (Melzacka et al 1979; Caccia et al 1981 a,b) which has several pharmacological activities; it is anorectic (Samanin et al 1979) analgesic (Rochat et al 1982) and anti-withdrawal in morphine-tolerant rats (Cervo et al 1981). Furthermore CPP shows effects on the central 5-hydroxytryptaminergic system compatible with agonistic activity on 5-HT postsynaptic receptors (Samanin et al 1979; Rokosz-Pelc et al 1980). CPP was also found in rats after oral administration of trazodone. It accumulates in the brain at concentrations comparable to those found after the administration of pharmacologically and biochemically effective doses of CPP (Caccia et al 1981b; Cervo et al 1981). Independent studies by Maj's group indicated that trazodone has biphasic action on the central 5-HT-ergic mechanisms; at lower doses the drug displays antagonist properties and at high doses an agonistic effect predominates (Maj et al 1979). The latter effect has been attributed to the formation of CPP (Maj et al 1980).

All these findings suggest that CPP contributes to some extent to the therapeutic effect of trazodone in depressed patients. To support this hypothesis it must first be demonstrated that CPP is actually formed in man treated with trazodone.

*Methods*

Four healthy volunteers, from 21 to 30 years and from 49 to 80 kg, participated in a study. After an overnight fast, subjects ingested a single dose (150 mg) of trazodone hydrochloride and blood samples were drawn over 10 h. Blood samples were centrifuged to separate plasma and stored at −20 °C. Urine was collected for 24 h dosing and hydrolysed with β-glucoronidase-arylsulphatase. Male CD-COBS rats, ca 200 g (Charles River, Italy), were orally dosed with 25 mg $kg^{-1}$ of trazodone hydrochloride. Concentrations of trazodone and CPP in plasma, brain and urine (5 ml) were determined by gas liquid chromatography as previously described (Caccia et al 1981a).

*Results*

Small amounts of trazodone were found in the 24 h urine of volunteers (0·5 ± 0·3% of the administered dose) in agreement with previous reports (Catanese & Lisciani 1970; Yamato et al 1976) that the drug is almost completely eliminated from the body by biotransformation. CPP was present in the urine of all four subjects as a minor metabolite amounting to only 0·15 ± 0·05% of the trazodone administered dose. Specificity of the

Table 1. Mean plasma concentrations of trazodone (Tz) and 1-(3-chlorophenyl)piperazine (CPP) after a single 150 mg dose of trazodone hydrochloride to 4 human subjects. * Mean 3 subjects.

| Time (h) | Plasma concentrations (nmol $ml^{-1}$ ± s.e.m.) | |
|---|---|---|
| | Tz | CPP |
| 1 | 4·66 ± 0·46 | 0·03 ± 0·01* |
| 2 | 5·24 ± 0·38 | 0·05 ± 0·02 |
| 4 | 3·21 ± 0·58 | 0·06 ± 0·02 |
| 6 | 2·19 ± 0·30 | 0·05 ± 0·02 |
| 8 | 1·52 ± 0·08 | 0·04 ± 0·01* |
| 10 | 1·03 ± 0·17 | 0·03 ± 0·01* |

* Correspondence.