Effect of trazodone on brain dopamine metabolism

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Trazodone 2- 3- 4(*m*-chlorophenyl) l-piperazinyl-propyl s-triazolo 4,3-a-pyridin-3-(2H)one is a new psychotropic agent with antidepressant, anti-anxiety and analgesic action (Hollister & Overall, 1965; Cianchetti & Gainotti, 1968; Cioffi, Mattiolo & others, 1969; Silvestrini & Quadri, 1970; Altissimi, Mastrostefano & Signore, 1971; Reggiani, Martini & Dionisio, 1972; Ban, Amin & others, 1974).

In animal experiments, trazodone shares various pharmacological effects with neuroleptics; both produce sedation, inhibit avoidance-conditioned reflexes, protect against amphetamine-induced lethality in aggregated mice and inhibit the peripheral effects of adrenaline and 5-HT (Silvestrini, Cioli & others, 1968; Boissier, Portmann-Cristesco & others, 1974).

However, unlike classic neuroleptics, trazodone does not produce catalepsy, even at subtoxic doses (Silvestrini & others, 1968), does not antagonize amphetamine- or L-dopa-induced stereotypies in rats (Silvestrini & Lisciani, 1973), or the emetic response to apomorphine in dogs (Silvestrini, 1975). On the other hand, trazodone has in common with narcotic analgesics the property of inhibiting the response to painful or unpleasant stimuli in rats (Silvestrini & others, 1968).

The above findings led us to investigate whether trazodone shares with neuroleptics and narcotic analgesics (Andén, Ross & Werdinius, 1964; Laverty & Sharman, 1965; Sasame Perez-Cruet & others, 1972), the property of stimulating dopamine synthesis.

The present study was on male Sprague-Dawley rats, 150–180 g, and male albino mice, 18–22 g. Animals were kept at 23° with the lights on from 8:00 to 20:00. They had free access to food and water.

Drugs were dissolved in twice-distilled water and injected intraperitoneally. The timing of the injections was calculated so that all animals were killed between 09.00 and 11.00 a.m.

Rats and mice were decapitated, their brains were rapidly frozen in liquid nitrogen and stored at —20°, Brain dopamine was extracted according Balakleewsky & Tagliamonte (1971) and assayed according to Laverty & Taylor (1968), homovanillic acid (HVA) according to Biggio & Piccardi (1973), dihydroxyphenylacetic acid (Dopac) according to a modification by Di Chiara & Porceddu (in preparation) of the methods of Murphy, Robinson & Sharman (1969) and Spano & Neff (1971), 5-HT and 5-hydroxyindolacetic acid (5-HIAA) by the method of Curzon & Green (1970).

Trazodone increased HVA and Dopac concentrations in the rat brain. A 96 % increase in the HVA content was produced by a dose of 25 mg kg^{-1}, while a 5-fold increase was observed after 100 mg kg^{-1}, the maximal tolerated dose of the compound. The effect on the Dopac concentration was less pronounced: an increase of 56 % was obtained with a dose of 25 mg kg^{-1} while 100 mg kg^{-1} of trazodone increased the Dopac concentration by 150%. On the other hand, trazodone did not influence dopamine concentrations at all doses used (Table 1).

After doses of the drug of 50 mg kg^{-1} or higher, rats appeared sedated, but did not exhibit catalepsy.

Trazodone-induced changes on HVA concentration were of short duration. After 50 mg kg^{-1} in rats, HVA and DOPAC concentrations reached a peak within 60 min and returned to normal 3 h after drug administration. The sedative response followed a similar time course.

Analogous results were obtained in mice: 1 h after treatment, trazodone (50 mg kg^{-1}, i.p.) raised HVA and Dopac concentrations, by 80 and 40%, respectively, but did not influence dopamine concentration. In mice, as in rats, the effect on dopamine metabolites is short-lasting.

Naloxone (1 mg kg^{-1}), a specific morphine antagonist, failed to prevent the rise in brain HVA induced by trazodone, while as expected from previous studies (Kuschinsky & Hornykiewicz, 1972), it antagonized the morphine-induced accumulation of HVA in the mouse brain (Table 2).

This study indicates that trazodone shares with butyrophenone and phenothiazine neuroleptics and with narcotic analgesics the capacity of increasing brain dopamine metabolites.

It is likely that this effect is secondary to an increased turnover of dopamine and not to an unspecific inhibition of the outflow of the acid metabolites from the brain. In fact, we have confirmed previous studies

Table 1. *Effect of trazodone on the concentrations of dopamine, HVA and Dopac in the rat brain.*

Trazodone mg kg^{-1}	Dopamine ng g^{-1}	HVA ng g^{-1}	%	Dopac ng g^{-1}	%
0 (18)	750 ± 14·8	52 ± 3·1	100	151 ± 5·7	100
25 (7)	783 ± 15·1	102 ± 4·3**	196	236 ± 3·0*	156
50 (11)	761 ± 16·2	148 ± 4·9**	284	289 ± 7·5**	191
100 (7)	757 ± 14·9	261 ± 8·9**	501	378 ± 8·6**	250

Each value is the mean ± s.e. of the number of experiments. ( ).
Animals were killed 1 h after treatment.
* $P < 0.01$ with respect to control value.
** $P < 0.001$ with respect to control value.

* Correspondence.

Table 2. *Failure of naloxone to prevent the increase in HVA concentrations produced by trazodone in the mouse brain.*

Treatment i.p.	Dose mg kg^{-1}	Min before death	HVA ng g^{-1}
Saline		50	146 ± 6·3
Trazodone	50	50	262 ± 9·2*
Naloxone	1	65	152 ± 6·9
Naloxone + trazodone	1 + 50	65 + 50	282 ± 9·1*
Morphine	10	50	295 ± 10·0*
Naloxone + morphine	1 + 10	65 + 50	165 ± 7·1

Each value is the mean ± s.e. of 4 determinations.
* $P < 0.01$ with respect to control value.

(Yamatsu, Kaneko & others, 1974; Angelucci & Bolle, 1974) that the drug does not raise, but actually causes a modest decrease (23%) in brain 5-HIAA content.

It is possible that the increase in dopamine synthesis found after trazodone administration is a compensatory feedback response to the blockade of dopamine receptors. Indeed, a similar theory has been proposed both for neuroleptics (Nyback & Sedvall, 1968); and for methadone (Sasame & others, 1972).

However, the effect of trazodone differs from that of these compounds in that trazodone does not cause catalepsy, and from the effect of narcotic analgesics in that the effect of trazodone is not antagonized by naloxone.

The reason for the dissociation of the increased dopamine turnover from the cataleptogenic effect might lie in the fact that the trazodone-induced blockade of dopamine from nerve terminals is overcome by the increased liberation of dopamine from nerve terminals.

A similar hypothesis has been suggested to explain a like dissociation following the administration of clozapine (Bartholini, Haefely & others, 1972).

Alternatively, it is possible that the trazodone-stimulation of dopamine synthesis is independent of the blockade of post-synaptic dopamine receptors. In fact, a similar hypothesis has been suggested for another drug, sulpiride, which also stimulates dopamine turnover without causing catalepsy (Tagliamonte, De Montis & others, 1975). One might suggest that these compounds block the postulated pre-synaptic receptors at the dopamine nerve terminals (Carlsson, 1975), thereby preventing the inhibitory control by dopamine on its own synthesis.

At the present time, it is not clear if the biochemical changes induced by trazodone in the rat or mouse brain have some relevance in any of the various therapeutic effects of trazodone in man.

This study was supported by Angelini S.p.A., Rome, Italy.

March 30, 1976

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Gas chromatographic-mass spectrometric identification and determination of residual by-products in clofibrate preparations

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Three products of clofibrate (ethyl-2-(4-chlorphenoxy)-2-methylpropionate), which are used in the treatment of hyperlipidemia, can be purchased in Sweden. In a clinical study, the effects of two clofibrate products on plasma lipid-concentration were compared (Olsson Orö & Rössner, 1974). The results revealed that the two products had the same effect against hyperlipidemia and no difference in the frequency of side-effects could be noticed. In an earlier study at this laboratory the three clofibrate preparations were analysed by gas chromatography (Mårde & Ryhage, 1975). The total amount of impurities in each product did not exceed the maximum accepted concentration of 1·5 ‰ (Pharmacopoea Nordica, 1973). Impurities in clofibrate have recently been studied by gas chromatography-mass spectrometry (Diding, Sandström & others, 1976). Three main impurities were found, the methyl ester analogue of clofibrate its deschloro analogue and the dichloro analogue.

We have used gas chromatography-mass spectrometry (g.c.-m.s.) for identifying other impurities in clofibrate preparations.

Samples of three drug preparations (I, II and III) from three manufacturers were obtained by dissolving 0·5 ml of capsule-content in 0·5 ml chloroform. 5 μl of the sample was injected into the combined gas chromatograph-mass spectrometer LKB 2091. The g.c.-column used was a 3 % SE-30 glass column 2·7 m × 2 mm (i.d.). A constant temperature of 160° for the first 8 min, followed by temperature programming at a rate of 10° min^{-1} was used. The carrier gas flow rate was 25 ml helium min^{-1}. The mass spectra were obtained with a constant accelerating voltage of 3·5 kV, an electron energy of 70 eV and an ionizing current of 100 μA. The g.c.-m.s. instruments was connected to the LKB 2130 data system and repetitive scanning of the mass range 10 to 500 in 2 s was used (Hedfjäll & Ryhage, 1975).

One of the analysed samples (I) is shown in Fig. 1, which represents a gas chromatogram obtained by using a flame ionization detector and isothermal operation of the column in accordance with instructions for analysis of clofibrate preparations (Mårde & Ryhage, 1975; Pharmacopoea Nordica, 1973). By using this standard method in drug purity control, impurities having a longer retention time than clofibrate itself will not be detected and due to the high concentration of clofibrate, neither will impurities having the same retention time be detected. Identification of the different components is not required.

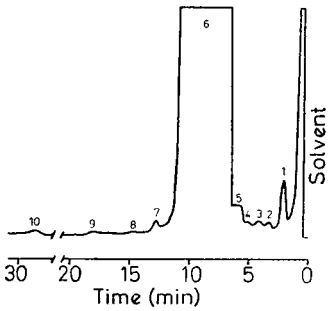

FIG. 1. Gas chromatographic analysis of clofibrate-preparation I where peak 6 is clofibrate.

The three samples were studied by g.c.-m.s. and Fig. 2 shows the total ion current (TIC) diagram of the same sample as shown in Fig. 1 where the corresponding

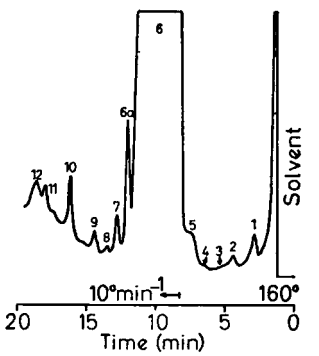

FIG. 2. Gas chromatographic-mass spectrometric analysis of clofibrate-preparation I.

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