Influence of the dopamine-β-hydroxylase inhibitor FLA 63 on the disposition of barbitone in the mouse

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FLA 63 bis(1-methyl-4-homopiperazinylthiocarbonyl) disulphide is generally recognized as a potent inhibitor of the enzyme dopamine-β-hydroxylase (DβH). FLA 63 was employed in the recent past as a pharmacological tool to assess the role of brain dopamine and noradrenaline in the actions of some centrally acting drugs such as amphetamine and morphine (Corrodi et al 1970; Reinhold et al 1973; Paalzow & Paalzow 1975). However, FLA 63 was shown to alter the tissue distribution of amphetamine in the rat, and it has been suggested that conclusions based solely on the principle that FLA 63 inhibits DβH must be reconsidered (Jonsson & Lewander 1974). FLA 63 has a chemical structure that is closely related to that of disulfiram, another inhibitor of DβH. Recently, it was found that in the rat disulfiram enhances the sleeping time of barbitone, alters its distribution in various tissues and reduces its urinary elimination (Sharkawi & Cianflone 1978).

In this communication, experiments are described that indicate that FLA 63 can alter the tissue distribution of barbitone, impair its renal elimination and enhance its pharmacological activity.

Male mice (Charles River CD-1), 25–35 g, housed in temperature-regulated quarters (23–25 °C) on 12-h light-dark cycle (lights on from 7:00 to 19:00) and having free access to food and water were used. Groups of 8 mice each received 10 ml kg^{-1} of 1% carboxymethylcellulose (CMC) intraperitoneally or FLA 63, 40 mg kg^{-1}, i.p., suspended in 1% solution of CMC. One hour later all groups were given an i.p. injection of barbitone sodium, 100 mg kg^{-1}. At different times (2, 4, 8 h) after barbitone administration, animals were decapitated and blood was collected in 5 ml test tubes containing about 500 units of heparin in 0·1 ml of 0·9% NaCl solution. The blood was then immediately analysed for barbitone content. The brain, liver, and kidneys were immediately removed after decapitation and stored at —12 °C. Mice in the 8 h groups were placed in pairs in metabolic cages, 23 × 25 × 18 cm, to allow for urine collection. The barbitone content of blood, brain, liver, kidneys and urine collected during the 8 h period was measured spectrophotometrically as described previously (Butler 1950). This method involves extraction of barbitone into diethylether from blood or urine diluted with phosphate buffer (pH 6), or from tissue samples homogenized in the same buffer. An aliquot of the ether layer is aspirated. The barbitone in the aliquot is then extracted with phosphate buffer (pH 11) and its optical density in the buffer is measured. The concentration of barbitone is found from a standard curve relating optical density to concentration. Percentage recoveries varied between 85 and 89%. The specificity of this was proven by the use of liquid scintillation spectrometry to measure [^{14}C]barbitone in some biological samples where similar results were obtained.

As seen in Fig. 1A, FLA 63-treated animals had a significantly higher ($P<0·05$) concentration of barbitone in blood, brain, liver, and kidneys, at the times examined. The amounts of barbitone excreted in urine during an 8 h period by FLA 63-treated animals are significantly less ($P<0·01$) than by CMC-treated mice as seen in Fig. 1E.

In addition, the same dose of FLA 63 (40 mg kg^{-1}, i.p.) 1 h before barbitone sodium (300 mg kg^{-1} i.p.) was found to markedly increase the sleeping time of barbitone in mice (barbitone—CMC, 60 ± 10 min; barbitone—FLA 63, more than 600 min).

The marked prolongation of barbitone sleeping time appears to result from changes in its disposition. This is indicated by the fact that, at the 3 times at which animals were killed, the concentrations of barbitone in blood, brain, liver and kidneys were significantly

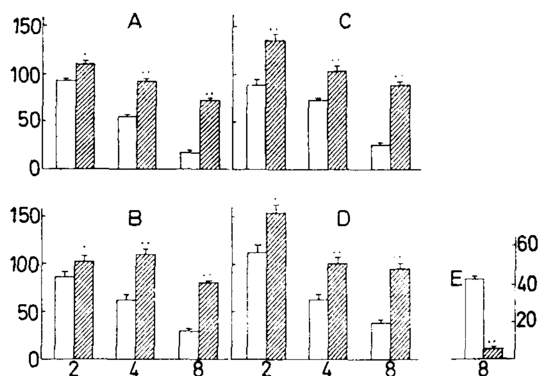

FIG. 1. The concentration of barbitone (left ordinate: mg g^{-1} or mg ml^{-1}) in blood (A), brain, (B), liver (C) and kidneys (D) in CMC- and FLA 63-treated mice at different times after the administration of barbitone sodium 100 mg kg^{-1} i.p. The amount of barbitone excreted in urine (right ordinate) in 8 h is calculated as % of the dose administered (E). CMC or FLA 63 were given i.p. 1 h before the administration of barbitone sodium: open columns, CMC-treated, hatched columns, FLA 63-treated. Each value represents the mean ± standard error of the mean obtained from a different group of 8 mice each. Abscissa: time (h).

* Statistically significant ($P<0·05$) by two-tailed Student's t-test.
** Statistically significant ($P<0·01$) by two tailed Student's P-test.

higher in FLA 63-treated animals compared with control animals. These increases in tissue concentrations could result from the markedly decreased renal elimination of barbitone in FLA 63-treated animals, particularly as barbitone is known to be excreted almost entirely in the urine without undergoing significant biotransformation (Williams 1959).

In 1974, Jonsson & Lewander showed in the rat that FLA 63 significantly increased amphetamine-induced increase in motor activity. They also found that pretreatment with FLA 63 was associated with a significant increase of amphetamine concentration in brain and plasma. Recently we found that disulphiram markedly enhanced amphetamine toxicity in the rat (Sharkawi et al 1978).

FLA 63 is considered as a more specific inhibitor of DβH than disulfiram. However, the present experiments indicate that FLA 63 affects barbitone disposition and pharmacological activity in a manner similar to that produced by disulfiram (Sharkawi & Cianflone 1978). Unpublished experiments from this laboratory show that both FLA 63 and disulfiram prolong morphine-induced analgesia in the rat and prolong sleep induced by chloral hydrate and phenobarbitone in mice. Thus, it seems that FLA 63 has a pharmacological profile that is similar to disulfiram. This should not seem surprising since both compounds are closely related chemically.

The present experiments and those of Jonsson & Lewander (1974) indicate that FLA 63 can alter the disposition of some centrally acting drugs. Consequently, their pharmacological activity is altered. Thus, it seems prudent that this effect of FLA 63 on drug disposition and elimination should be taken into consideration when FLA 63 is employed as an inhibitor of DβH to assess the role of dopamine and noradrenaline in the pharmacological actions of certain centrally acting drugs.

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The effects of Δ^9-tetrahydrocannabinol, cannabidiol, and shock on plasma corticosterone concentrations in rats

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Barry & Buckley (1966) have reported that in rats, stress caused a pituitary-adrenal activation, as measured by increased plasma corticosterone concentrations. Furthermore, Kubena et al (1971) demonstrated that in rats Δ^9-tetrahydrocannabinol (Δ^9-THC) in doses of 2–16 mg kg^{-1} also caused pituitary-adrenal activation. This was corroborated by Kokka & Garcia (1974) for 5–20 mg kg^{-1} doses of Δ^9-THC. Graham & Li (1973) found that cannabidiol, another major naturally occurring cannabinoid, had no observed effects on either the cardiovascular or respiratory systems of the rat.

Other studies in this laboratory have examined the action of Δ^9-THC on several endocrine organs including the gonads (List et al 1977) and the thyroid (Nazar et al 1977). Our latest study was designed to examine the effect of Δ^9-THC and CBD on the normal pituitary-adrenal activation response to stress.

Male albino rats (Camm Wistar strain), 175–325 g, were housed separately and maintained at constant room temperature 20 °C on a 12 h light–12 h dark schedule with free access to food and water. The test procedures were performed during the lighted phase. The Δ^9-THC and CBD were obtained from the National Institute of Mental Health and suspended in 1% Tween 80, 10% propylene glycol, and 89% physiological saline (0·9% NaCl) for injection. The control injection contained the same volumes of Tween 80, propylene glycol, and saline as were included in the highset dose of Δ^9-THC or CBD in each experiment. Δ^9-THC and CBD injected animals received 5 mg kg^{-1} doses. All injections were intraperitoneal and in a volume of less than 1 ml. Electric shocks of 60 V (5 shocks, of 3 s duration with 9 s interval) were administered over 1 min 15 min before decapitation and collection of trunk blood. Serum samples were assayed fluorometrically for plasma corticosterone, as described by Perhach & Barry (1970), 1 h after injection of cannabinoid.

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