The effects of barbiturates on the activity of the catecholamine neurones in the rat brain

SIR,—Previous biochemical and histochemical studies have demonstrated the importance of neuronal impulse flow for the disappearance of catecholamines and 5-hydroxytryptamine after inhibition of their biosynthesis (Fuxe & Gunne, 1964; Dahlström, Fuxe, Kernell & Sedvall, 1965; Andén, Corrodi, Dahlström, Fuxe & Hökfelt, 1966; Corrodi & Malmfors, 1966). The methyl ester of α-methyltyrosine (H 44/68) has been found by ourselves to be a potent inhibitor of catecholamine synthesis without affecting the uptake-storage mechanism of the intraneuronal granules (Andén & others, 1966; Corrodi, Fuxe & Hökfelt, 1966; Corrodi & Hanson, 1966). This substance could therefore be used to reveal any changes that may occur in the activity of the brain catecholamine neurones under the influence of pharmacological, physiological or experimental conditions. This approach was originally suggested to us by Hillarp a few years ago. A decrease in impulse flow would thus have the effect of a decreased rate of disappearance of the catecholamines from the axon terminals after treatment with H 44/68, while an increased impulse flow would increase the rate of disappearance of the catecholamines after inhibition of synthesis. In the present study the effects of barbiturates on the activity of the catecholamine neurones were studied in two experimental situations. In the first experiment a single intraperitoneal injection of pentobarbitone sodium, 40 mg/kg, was given to male Sprague-Dawley rats (150–250 g). The animals at an environmental temperature of 25° were kept heavily sedated, without righting reflex, by repeated injections of small amounts (10–15 mg/kg). Fifteen min after the first injection the animals were treated with H 44/68 (250 mg/kg. i.p.) and were killed by decapitation 4 or 8 hr later. Control animals received either pentobarbitone or H 44/68 in the same way as the experimental animals.

In another experiment phenobarbitone sodium was used. The rats which were killed 4 hr after H 44/68 received a large dose of phenobarbitone sodium (100 mg/kg i.v.), 15 min before H 44/68 administration (250 mg/kg i.p.) and a small dose (40 mg/kg. i.p.) 2 hr before killing. This treatment made the rats sleep deeply. The rats which were killed 8 hr after the H 44/68 injection received two large doses of phenobarbitone sodium (100 mg/kg i.v., 8 hr 15 min and 4 hr before killing) and two small doses of the drug (40 mg/kg i.p. 6 and 2 hr before killing). Control groups were treated in the same way as in the previous experiment. The rectal temperature was recorded frequently and found to be normal in all animals.

For the histochemical investigation defined parts of the brains were dissected, freeze-dried, treated with formaldehyde gas, embedded and sectioned using the histochemical fluorescence method developed by Hillarp (Falck, Hillarp, Thieme & Torp, 1962; see review by Hillarp, Fuxe and Dahlström, 1966). Whole brains were removed and analysed for dopamine and noradrenaline (Bertler, Carlsson & Rosengren, 1958; Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962).

The biochemical determinations (see Tables 1 and 2) demonstrated that the brains of rats treated with barbiturates showed a significant decrease in the rate of disappearance of dopamine both 4 and 8 hr after H 44/68 treatment compared to rats treated with H 44/68 alone. The rate of disappearance of brain noradrenaline, however, was not much affected by the barbiturate administration. No certain changes were obtained in the amine levels of the catecholamine neurones after treatment with barbiturates alone.

With the aid of the histochemical fluorescence method it was found that the

dopamine nerve terminals of the nucleus caudatus putamen, nucleus accumbens, the tuberculum olfactorium and the median eminence of rats treated with barbiturate and H 44/68 had a greater fluorescence intensity than those of animals treated with H 44/68 alone. The difference in intensity was more evident 8 hr than 4 hr after H 44/68 treatment. The central noradrenaline nerve terminals of various areas, for example, the hypothalamus, and the neocortex, of animals treated with barbiturates and H 44/68 or with H 44/68 alone did not show any clear difference in the degree of depletion obtained 4 and 8 hr after administration of the drugs. The same was also true for the central dopamine and noradrenaline cell bodies. No obvious change in the appearance of the central catecholamine and 5-hydroxytryptamine neurones was obtained after treatment with barbiturates only.

TABLE 1. THE CONTENT OF NORADRENALINE AND DOPAMINE IN RAT BRAIN AFTER PENTOBARBITONE AND H 44/68 EXPRESSED AS A PERCENTAGE OF NORMAL VALUES ± S.E.M. OF 4 EXPERIMENTS
(Dosage and treatment see text)

Treatment		Noradrenaline (%)	Dopamine (%)
Nontreated		100·0 ± 2·5	100·0 ± 3·8
Pentobarbitone	4 hr	93·8 ± 6·7	98·6 ± 4·4
H 44/68	4 hr	45·9 ± 0·8	*26·3 ± 1·4
Pentobarbitone + H 44/68	4 hr	55·5 ± 1·5	*45·0 ± 0·6 (*P = 0·001)
Pentobarbitone	8 hr	104·1 ± 3·4	98·1 ± 4·5
H 44/68	8 hr	32·9 ± 1·3	*12·9 ± 0·2
Pentobarbitone + H 44/68	8 hr	34·6 ± 0·5	*30·8 ± 1·9 (*P = 0·001)

TABLE 2. THE CONTENT OF NORADRENALINE AND DOPAMINE IN RAT BRAIN AFTER PHENOBARBITONE AND H 44/68 EXPRESSED AS A PERCENTAGE OF NORMAL VALUES ± S.E.M. OF 4 EXPERIMENTS
(Dosage and treatment see text)

Treatment		Noradrenaline (%)	Dopamine (%)
Nontreated		100·0 ± 2·5	100·0 ± 3·8
Phenobarbitone	4 hr	98·3 ± 3·7	104·1 ± 3·1
H 44/68	4 hr	44·5 ± 3·3	*24·2 ± 0·8
Phenobarbitone + H 44/68	4 hr	50·0 ± 1·0	*44·7 ± 4·2 (*P < 0·005)

Thus, both the histochemical and biochemical experiments support the view that pentobarbitone and phenobarbitone induce a markedly decreased impulse flow in the various dopamine neurones, with a subsequent decreased rate of synthesis in these systems. This has the effect of slowing up of the depletion obtained by an inhibition of dopamine synthesis. In other words, the dopamine neurones are in a lower state of activity than normal during barbiturate sleep. It is not known whether this effect is indirect or direct. The noradrenaline neurone systems, however, were hardly affected by the barbiturates.

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Concerning the regulation of some diverse biochemical reactions underlying the inflammatory response by salicylic acid, phenylbutazone and other acidic antirheumatic drugs

SIR,—Under this title, we proposed the hypothesis that lysyl ϵ-amino-groups of certain proteins are important binding sites for acidic anti-inflammatory drugs, and that when so bound these drugs may inhibit enzymic reactions which depend upon the availability of these ϵ-amino-groups—either for binding an essential cofactor (for example pyridoxal phosphate) or for directing the enzymic reaction (for example tryptic-like proteolysis or mitochondrial phosphorylation) (Whitehouse & Skidmore, 1965). We now have evidence that these particular drugs may also influence other biochemical reactions which might be involved in the inflammatory response, by acting as pseudo-antimetabolites and inhibiting the metabolism of certain derivatives of aromatic amino-acids.

Enzymes resembling chymotrypsin (E.C. no. 3.4.4.5) in their substrate specificity have been found in the granules of rat mast cells (Lagunoff & Benditt, 1963) and have been implicated in the swelling of the paw of the rat after the local injection of an inflammatory agent (Hladovec & Rybák, 1963) and also in the anaphylactic release of histamine either from sensitised lung of the guinea-pig (Austen & Brockelhurst, 1961a) or from rat peritoneal mast cells (Keller, 1963). Chymotrypsin hydrolyses esters and amides of aromatic amino-acids, leucine, methionine and even histidine (Kloss & Schröder, 1964) as well as proteins, and releases histamine from mast cells with concomitant degranulation (Üvnas & Antonsson, 1963; Saeki, 1964). Sodium salicylate inhibits a guinea-pig lung protease (Ungar, Yamura, Isola & Kobrin, 1961) and the anaphylactic release of histamine in guinea-pigs (Mongar & Schild, 1957) and rabbits (Haining, 1956). Mörsdorf, Donner & Cornellisson (1966) found that the *N*-acetyltyrosine esterase present in the inflamed rat paw was powerfully inhibited by several acidic anti-inflammatory drugs.