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Modification of the responses of brain stem neurones to transmitter substances by anaesthetic agents

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Summary

1. The effects of microiontophoretic applications of acetylcholine (ACh), (−)-noradrenaline ((−)-NA) and 5-hydroxytryptamine (5-HT) have been investigated on spontaneously active brain stem neurones in decerebrate unanaesthetized rats and in rats anaesthetized wih either tribromoethanol, urethane or pentobarbitone.

2. Four types of responses to both (−)-NA and 5-HT were seen. These were: simple excitation; excitation preceded by a short-lasting inhibition; short-lasting inhibition and prolonged inhibition. Three types of responses to ACh were seen: an excitation with long latency of onset; excitation with short latency of onset, resembling the response to an excitant amino acid, and a short-lasting inhibitory response.

3. The types of responses to microiontophoretically applied ACh, (−)-NA or 5-HT in anaesthetized and unanaesthetized animals were similar.

4. The number of ACh excitatory responses with short latency of onset were significantly reduced in the pentobarbitone-anaesthetized group and a small but significant increase in the number of 5-HT inhibitory effects were observed in each anaesthetized group of animals.

5. A significantly greater proportion of slower firing neurones (less than 10 spikes/s) were found in the pentobarbitone-anaesthetized animals.

6. The effects of microiontophoretically applied and i.v. administered pentobarbitone were studied on spontaneously active neurones which responded consistently to ACh and a control agonist.

7. Pentobarbitone administered by either route reduced the firing rate of most neurones studied and was shown to antagonize specifically the excitation of neurones by exogenously applied ACh.

8. It is suggested that postsynaptic antagonism of endogenously released ACh may be a contributing factor in the mechanism of action of pentobarbitone.

Introduction

Many anaesthetic agents modify neurotransmission in the central nervous system and this modification has been studied by measuring changes in brain neurotransmitter levels (Richter & Crossland, 1949; Anderson & Bonnycastle, 1960; Corrodi, Fuxe & Hökfelt, 1966) and in release of transmitter substances from various areas of the central nervous system (C.N.S.) during anaesthesia (Mitchell,

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1963 ; Phillis & Chong, 1965 ; Phillis, 1968). Furthermore, anaesthetics have been shown to alter the sensitivity of neurones in the C.N.S. to various transmitter substances (Bloom, Costa & Salmoiraghi, 1965 ; Crawford, 1970 ; Johnson, Roberts & Straughan, 1969), effects which were not always appreciated in earlier neurophysiological investigations. Thus, Curtis, Eccles & Eccles (1957), using anaesthetized preparations, found little or no effect of adrenaline or noradrenaline on monosynaptic reflexes. Microiontophoretic applications of adrenaline, noradrenaline, 5-hydroxytryptamine and dopamine onto spinal neurones were found to be ineffective (Curtis, Phillis & Watkins, 1961 ; Curtis, 1962). It has since been shown that neurones in the spinal cord of unanaesthetized animals are sensitive to noradrenaline and 5-hydroxytryptamine (Weight & Salmoiraghi, 1966a, b ; Engberg & Ryall, 1966 ; Biscoe & Curtis, 1966) and therefore the earlier negative findings could be attributed to the use of barbiturate anaesthesia.

There is little evidence that the effects of anaesthetic agents in the C.N.S. are related to any single neurotransmitter. However, the responses of neurones to acetylcholine during barbiturate anaesthesia have received considerable attention, although the results from different studies are not always in agreement.

Depression of acetylcholine-induced excitation by anaesthetics has been reported to occur with neurones in the cortex (Krnjević & Phillis, 1963), thalamus (McCance, Phillis, Tebēcis & Westerman, 1968 ; Phillis & Tebēcis, 1967) and caudate nucleus (Bloom *et al.*, 1965). Reduction in the number of neurones found to be excited by acetylcholine in the olfactory bulb (von Baumgarten, Bloom, Oliver & Salmoiraghi, 1963) hypothalamus (Salmoiraghi & Stefanis, 1965) and in the cortex (Randić, Siminoff & Straughan, 1964) were considered to be due to barbiturate anaesthesia. On the other hand, several workers have been unable to demonstrate a selective action by barbiturates or other anaesthetics on acetylcholine excitation (Andersen & Curtis, 1964 ; Crawford & Curtis, 1966 ; Curtis & Ryall, 1966 ; Johnson *et al.*, 1969 ; Crawford, 1970).

There is evidence that sedatives and anaesthetic agents act on the brain stem reticular formation (Killam, 1962 ; 1968) and it has been proposed that depression of activity in the reticular formation is the basis of the anaesthetic state (French, Verzeano & Magoun, 1953). Single neurones in the brain stem have been shown to be sensitive both to systemic injections of small quantities of barbiturates (Rosina & Sotgiu, 1967) and to microiontophoretically applied pentobarbitone (Bradley & Wolstencroft, 1965).

Since the brain stem is considered to be an important site of action for some anaesthetic agents and since acetylcholine (ACh), (−)-noradrenaline ((−)-NA) and 5-hydroxytryptamine (5-HT) are thought to be the principal synaptic transmitters in this region of the C.N.S. (Bradley, 1968 ; Phillis, 1970), we investigated the effects of anaesthesia on neuronal sensitivity to these transmitter substances.

The responses of single neurones in the brain stem to microiontophoretic applications of ACh, (−)-NA or 5-HT were examined in rats anaesthetized with either tribromoethanol, urethane or pentobarbitone and compared with those from a similar study in unanaesthetized animals. In view of the results from pentobarbitone-anaesthetized animals, the study was extended to examine the effects of both microiontophoretically and intravenously administered pentobarbitone on neurones excited by ACh. Some of the results of this investigation have been communicated to the British Pharmacological Society (Bradley & Dray, 1972a).

Methods

Adult albino rats of 300–500 g were used throughout this investigation. Control animals were decerebrated and partially cerebellectomized under halothane anaesthesia (<0·5%), and the anaesthetic withdrawn 1–2 h prior to recording. Experiments on anaesthetized rats were performed on partially cerebellectomized animals in which anaesthesia had been induced with halothane and subsequently maintained with i.p. injections of a non-volatile anaesthetic. The anaesthetic agents used were tribromoethanol (Avertin, Bayer), 200 mg/kg as a 2·5% solution freshly prepared at 30° C; urethane (B.D.H. Ltd.), 1·8 g/kg administered in divided doses; pentobarbitone sodium (Nembutal, Abbott Labs. Ltd.), 50 mg/kg. The depth of anaesthesia was judged by the disappearance of both the corneal reflex and the hind-limb withdrawal reflex. Ten animals were used in each study.

Micropipettes were inserted through the dorsal surface of the medulla and pons between 1 and 4 mm rostral to the obex, between 1·5 mm either side of the mid-line and to a depth of 2·5 mm. Penetrations in the mid-line were avoided. Only spontaneously active neurones were studied.

Five-barrelled glass micropipettes with overall tip diameter 6–10 μm were used to record extracellular neuronal action potentials and to eject ions from solutions of drugs into the vicinity of the cell. Neuronal spikes were amplified and electronically counted as described by Bradley & Wolstencroft (1964).

The recording barrel of the pipettes contained 4 M saline. One barrel contained 1 M saline for monitoring the effects of iontophoretic current and the others contained drugs in aqueous solution. Drugs were usually ejected with a current of 50 nA at the appropriate polarity for one or more 5 s epochs. A retaining current of 15 nA was used to prevent leakage of compounds (Bradley & Candy, 1970). However, higher backing currents of up to 40 nA were often necessary to prevent leakage of amino acids.

The following drugs were used in the micropipettes at the indicated concentration after adjusting the pH as necessary: acetylcholine chloride, 10%, pH 4·0–5·0 (Hopkin & Williams or B.D.H. Ltd.); (−)-noradrenaline base, 10%, pH 4·5–5·5; DL-homocysteic acid, 10%, pH 8·0–9·0; glycine hydrochloride, 1·0%, pH 3·0–4·0 (Koch-Light); monosodium glutamate, 10%, pH 8·0–9·0 (L. Light & Co.); pentobarbitone sodium, 1–5%, pH 9·0–10·0 (Abbott Labs. Ltd.). Solutions of pentobarbitone (2–10 mg/kg) and thiopentone (2 mg/kg), (Abbott Labs. Ltd.) were administered via a polythene cannula inserted into the femoral vein.

The spontaneous neuronal firing frequencies and neuronal responses to microiontophoretic applications of ACh, (−)-NA or 5-HT were examined in rats anaesthetized with different anaesthetic agents and compared with those from unanaesthetized control animals. The actions of both microiontophoretically and i.v. administered barbiturate and the interactions with microiontophoretically applied transmitter substances on single brain stem neurones were also studied.

Results

In the present experiments most brain stem neurones tested with iontophoretically applied ACh responded with an increase in firing rate but occasionally a short-lasting inhibitory response was observed. The excitatory responses to ACh could be divided into two types according to the latency of onset. Type I excitation was

slow in onset (latency>5 s) and, like the inhibitory response, resembled the effects of ACh in the cat (Bradley, Dhawan & Wolstencroft, 1966). Type II ACh excitation, a new feature of ACh effects on brain stem neurones, occurred rapidly, with a latency of onset (0·2–0·8 s) similar to that seen with excitant amino acids. A more detailed description of the excitatory effects of ACh in the rat has been given elsewhere (Bradley & Dray, 1972b).

Four types of neuronal responses were observed with microiontophoretic applications of (−)-NA. These were: excitation, which was usually slow in onset and long-lasting; two types of inhibitory response, short- or long-lasting; and a biphasic response which consisted of excitation preceded by short-lasting inhibition. These responses were qualitatively similar to those reported for neurones in the cat brain stem (Boakes, Bradley, Brookes, Candy & Wolstencroft, 1971; Hösli, Tebēcis & Schönwetter, 1971). With 5-HT, long-lasting excitation of neurones was the predominant effect observed, although other responses to 5-HT, such as short- and long-lasting inhibition and a biphasic response, were also seen. These too, were qualitatively similar to those observed with cat brain stem neurones (Boakes, Bradley, Briggs & Dray, 1970).

Effects of anaesthetics on responses to acetylcholine, (−)-noradrenaline and 5-hydroxytryptamine

The anaesthetics used had no detectable effects on the types of responses elicited by iontophoretically applied ACh, (−)-NA or 5-HT. Thus, the characteristics of the responses observed with the three neurotransmitters were similar in each anaesthetized group and resembled those in the unanaesthetized control group. There were, however, differences in the proportions of the responses. The most striking difference observed was in the proportions of the responses in the pentobarbitone-anaesthetized group, where the number of ACh excitations with short latency of onset (Type II) was significantly reduced ($P<0·0005$), although the proportion of Type I ACh excitations, i.e. those with longer latency, remained unchanged (Table 1). Similar results were obtained in pentobarbitone-anaesthetized animals which were artificially ventilated to avoid hypoxia. In all anaesthetized groups a small but significantly greater number of neurones was inhibited by 5-HT.

From this comparative study it appeared that pentobarbitone had a marked effect on neuronal responses to ACh, especially Type II responses. Consequently the effects of pentobarbitone, applied either microiontophoretically or intravenously were examined on neurones which showed consistent responses to ACh (Type II) and another agonist.

TABLE 1. *Proportions of brain stem neurones in anaesthetized and unanaesthetized rats responding to microiontophoretic applications of acetylcholine, (−)-noradrenaline and 5-hydroxytryptamine*

	Acetylcholine					(−)-Noradrenaline				5-Hydroxytryptamine			
	+		O	−		+	O	−		+	O	−	
	I	II											
Tribromoethanol	22	57	19	2	(169)	41	32	27	(187)	71*	18	11*	(195)
Urethane	25	60	11	4	(311)	44	20*	36*	(201)	69	13	18*	(211)
Pentobarbitone	29	12*	50*	9*	(219)	51	25	24	(209)	79	12	9*	(212)
Unanaesthetized	20	61	16	3	(229)	42	32	26	(198)	81	16	3	(203)

The figures are percentages, the total number of neurones studied is given in parentheses in each case. The greatest difference is shown in the proportion of ACh II excitations found in the pentobarbitone-anaesthetized group, which are significantly reduced compared with the other groups. * Denotes $P<0·05$ (χ^2 test). +=excitation; O=no effect; −=inhibition.

Since urethane was well tolerated, provided long-lasting anaesthesia, and appeared to have little effect on the responses to exogenously applied ACh, (−)-NA or 5-HT, it was considered to be a satisfactory anaesthetic for use in the subsequent drug studies.

Effects of microiontophoretically applied pentobarbitone

Dilute solutions (1%) of pentobarbitone were used in the electrode in order to avoid any non-specific effects seen at higher concentrations (5%) such as depression of firing rate and marked reduction of the spike amplitude which makes counting impossible.

The effects of iontophoretically applied pentobarbitone were studied on 42 neurones. These neurones varied in their sensitivity to pentobarbitone and it was found that prolonged application depressed the spontaneous firing rate of 33 neurones and increased that of two. Antagonism of the excitatory effect of ACh was shown in 35 of the 42 neurones studied when pentobarbitone was applied for periods of 2–12 minutes. Of these 35 neurones, the spontaneous firing rate of 28 was depressed and of 7 was unaffected by pentobarbitone when ACh excitation was blocked. Some degree of recovery of the ACh response was seen in most studies 2–15 min after the termination of the pentobarbitone application. 5-Hydroxytryptamine, glutamate or DL-homocysteic acid were each used as control agonists with ACh. In many cases when pentobarbitone had antagonized excitatory effects of ACh (Fig. 1), excitation by 5-HT was unchanged (14 out of 20 neurones). Similar effects were observed with glutamate (20 out of 23 neurones) (Fig. 2) or DL-homocysteic acid (3 out of 4 neurones).

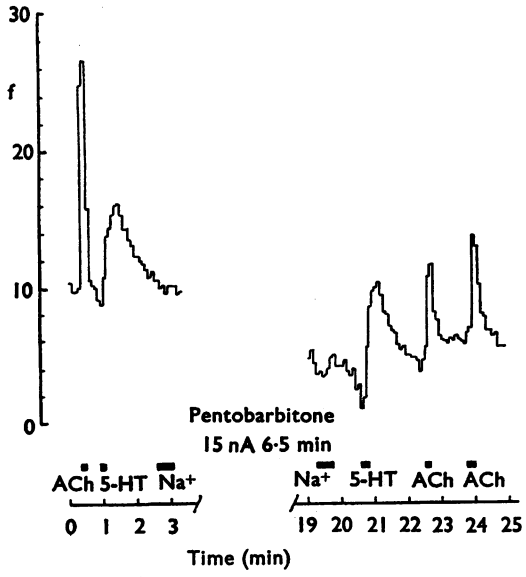

FIG. 1. The effect of microiontophoretic application of pentobarbitone on the responses of a spontaneously firing single brain stem neurone to acetylcholine (ACh) and 5-hydroxytryptamine (5-HT). The mean firing frequency (f) in impulses/s in successive 5 s periods is plotted against time in minutes. Iontophoretic applications of drugs or current control (Na^+) are shown by horizontal bars. The excitatory response to ACh but not that to 5-HT was reduced after applying pentobarbitone at 15 nA for 6·5 minutes. Unless otherwise indicated drugs were applied with a current of 50 nA.

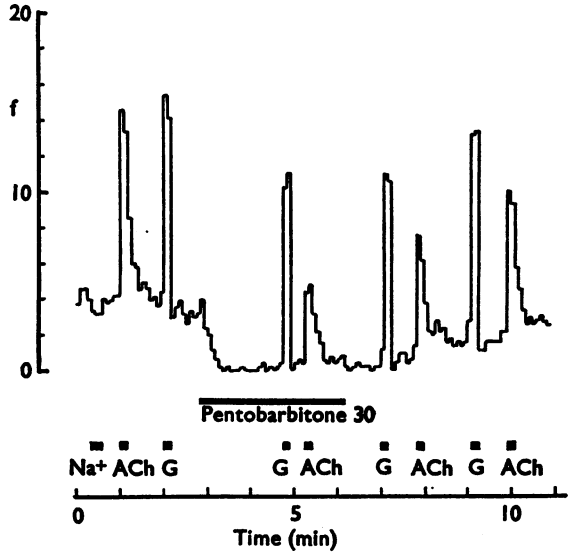

FIG. 2. The effect of iontophoretically applied pentobarbitone (30 nA) on the responses of a single brain stem neurone to acetylcholine (ACh) and glutamate (20 nA). Pentobarbitone depressed the spontaneous firing rate and the excitatory response to ACh but not that to glutamate (G). Partial recovery of the ACh response can be seen.

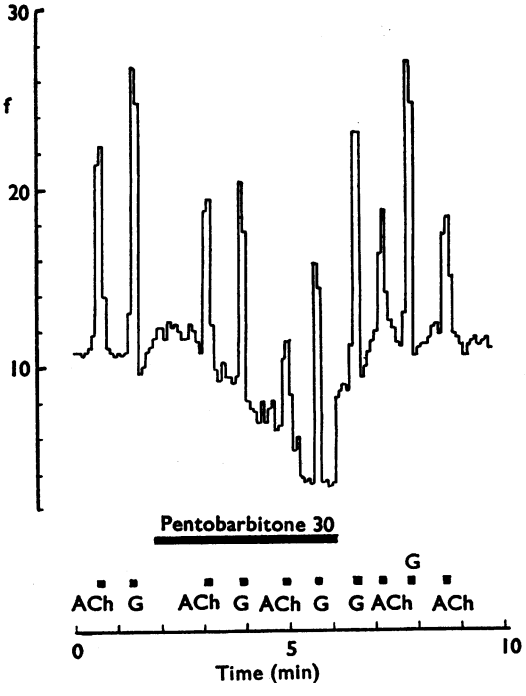

FIG. 3. The effect of microiontophoretic application of pentobarbitone (30 nA) on the responses of a brain stem neurone to acetylcholine (ACh) and glutamate (G). Pentobarbitone depressed the spontaneous firing rate and the excitatory response to ACh and glutamate (20 nA). The ACh response was reduced to a greater extent than that to glutamate and recovered more slowly.

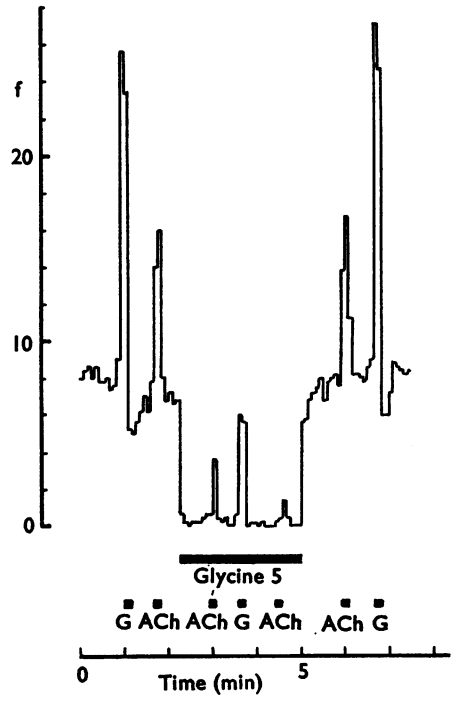

FIG. 4. The effect of microiontophoretic application of glycine (5 nA) on the responses of a single neurone to acetylcholine (ACh) and glutamate (G). Glycine depressed the spontaneous firing rate and the response to ACh and glutamate. Both depression and recovery of the excitatory responses occurred in parallel.

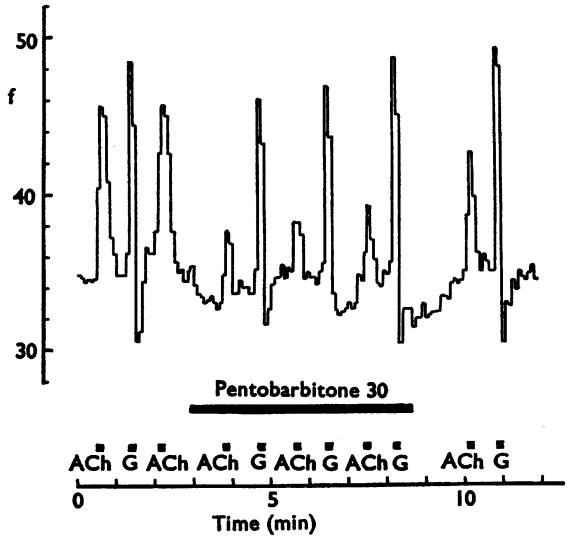

FIG. 5. Microiontophoretic application of pentobarbitone (30 nA) to a neurone excited by acetylcholine (ACh) and glutamate (G). The excitatory response to ACh but not glutamate was depressed but the spontaneous neuronal firing rate was unaffected.

Prolonged application of pentobarbitone depressed the firing rate of most neurones studied and occasionally, when the effect of a control agonist was also reduced, a clear-cut antagonism of ACh excitation was less readily seen (Fig. 3). However, the specificity of the action of pentobarbitone was demonstrated by the fact that the response to ACh was reduced more quickly or to a greater extent than that to the control agonist and the time course of the effects was different. Thus, the ACh effect always recovered more slowly than did the effect of the control agonist (Fig. 3). When the firing rate was depressed by a substance other than pentobarbitone, e.g. glycine, depression and recovery of the responses to ACh and the control agonist always occurred in parallel (Fig. 4). Antagonism of ACh excitation by pentobarbitone was also present when the spontaneous neuronal firing rate remained unchanged (Figure 5).

The effects of intravenous barbiturates

The effect of i.v. administration of small doses of pentobarbitone or thiopentone were studied on 10 neurones which showed consistent excitatory responses to ACh and a control agonist applied iontophoretically. The firing rate of 9 of the neurones studied was depressed after i.v. barbiturate, and that of 1 increased after thiopentone. A previous administration of saline of the same temperature, volume and pH as the barbiturate solution did not affect the neuronal firing rate or the responses to ACh and a control agonist. No change was observed in the extracellular spike potential after i.v. barbiturate or saline.

The excitatory response to ACh was reduced by pentobarbitone (1–5 mg/kg) in 5 out of 7 neurones studied, whereas excitation by 5-HT or glutamate was unaffected (Fig. 6). Antagonism of ACh excitation was observed 1–2 min after i.v. administra-

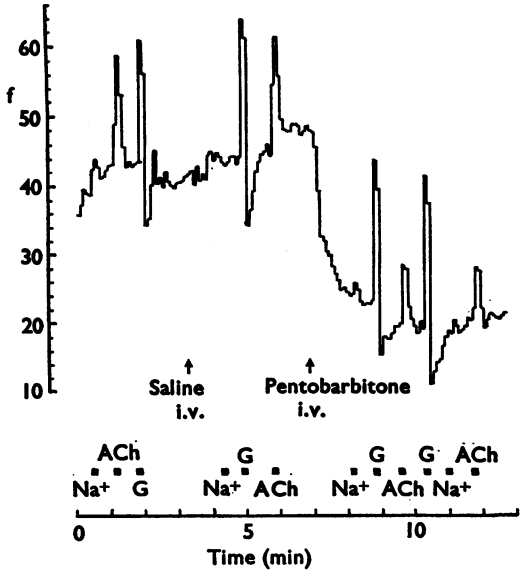

FIG. 6. The effect of intravenous (i.v.) pentobarbitone (10 mg/kg) on the responses of a single neurone to iontophoretically applied acetylcholine (ACh) and glutamate (G). Intravenous saline had no effect on the firing rate or the excitatory responses to ACh or glutamate. Pentobarbitone depressed the spontaneous firing rate and the excitatory response to ACh but not that to glutamate.

tion of pentobarbitone. Complete recovery was never seen even when the recording of neuronal activity was continued for 90 min after the injection of pentobarbitone. This drug had the same effect on the firing rate and the excitatory response to ACh in urethane-anaesthetized rats (3 neurones) as it did in unanaesthetized animals (4 neurones).

Thiopentone (1 mg/kg) antagonized the excitatory effect of ACh on each of 3 neurones studied. Excitation by 5-HT or DL-homocysteic acid, used as control agonists, was not affected (Fig. 7). Antagonism by thiopentone was demonstrated 0·5–1 min after its administration and some degree of recovery of the neuronal firing rate and the response to ACh occurred within 20 min of the thiopentone administration. However, complete recovery was not seen up to 50 min later. Since neurones were never found to recover completely after an i.v. injection of barbiturate, only one study was attempted in each animal.

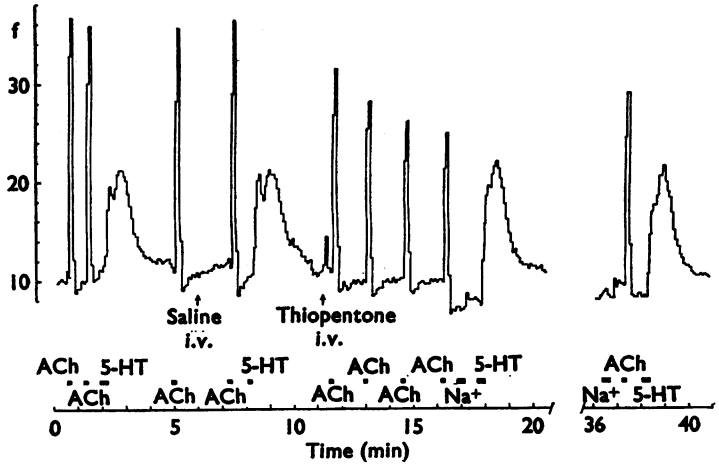

FIG. 7. The effect on intravenous (i.v.) thiopentone (2 mg/kg) on the responses of a single brain stem neurone to microiontophoretically applied acetylcholine (ACh) and 5-hydroxytryptamine (5-HT). Saline had no effect on the responses to ACh or 5-HT. After thiopentone the excitatory response to ACh was reduced, while that to 5-HT was unaffected. Some degree of recovery of the ACh excitatory response was seen 17 min later.

One of the characteristic actions of pentobarbitone applied either iontophoretically or i.v. was its depression of the spontaneous neuronal firing rate. In fact, in the initial comparative study a greater number ($P<0·01$) of slower firing neurones (less than 10 spikes/s) was observed in the group of animals anaesthetized with pentobarbitone compared with any of the other groups. In the tribromoethanol- and urethane-anaesthetized animals, however, the neuronal firing frequencies were not significantly different from those in unanaesthetized controls. The depression of neuronal firing by pentobarbitone was however unrelated to its specific antagonism of the excitation produced by exogenously applied ACh.

Discussion

Four types of response to iontophoretically applied (−)-NA and 5-HT have been found in single brain stem neurones in the rat. These were: (a) a simple excitatory response, (b) excitation preceded by an inhibitory phase, (c) short-lasting

inhibition and (d) an inhibitory response of long duration. These responses were qualitatively similar to those described for (−)-NA and 5-HT in previous studies on cat brain stem neurones (Boakes *et al.*, 1970, 1971 ; Hösli *et al.*, 1971). On the other hand, microiontophoretically applied ACh was found to excite most of the neurones studied and inhibition was less frequently observed. Furthermore, neurones which were excited by ACh were of two types, those excited after a long latency, and those rapidly excited, i.e. with a latency similar to that seen with excitant amino acids. The long latency response to ACh was similar to the excitatory responses observed with this substance in the cat (Bradley *et al.*, 1966) but the faster ACh responses have not previously been found. These responses to ACh have been described elsewhere (Bradley & Dray, 1972b). The possibility of any of the effects of iontophoretically applied substances being artifactual was eliminated by careful controls of current and pH effects.

The types of responses observed with exogenously applied ACh, (−)-NA or 5-HT were similar in each group of animals anaesthetized with one of three non-volatile anaesthetic agents and resembled those from the unanaesthetized control group. The most striking difference between the groups was in that anaesthetized with pentobarbitone, where the number of excitatory responses to ACh was significantly reduced (Table 1), especially excitations of short latency. Since a similar reduction in the number of ACh excitations was observed in artificially ventilated animals, it seems unlikely that hypoxia, due to the respiratory depression induced by pentobarbitone, could account for this effect. The significance of the reduction in the number of ACh excitatory responses of rapid onset is not clear at the present time but may reflect a greater sensitivity of this particular population of brain stem neurones to pentobarbitone.

Although a small but significant increase in the number of neurones inhibited by 5-HT was observed in each anaesthetized group, the relative infrequency of 5-HT inhibitory responses makes the interpretation of this finding difficult. Johnson *et al.* (1969) reported an increase in the number of inhibitory responses with monoamines applied iontophoretically to cortical neurones in anaesthetized animals but their observations were confined to pentobarbitone-anaesthetized animals where the number of neurones excited by ACh was similar to that in unanaesthetized preparations.

A reduction in the number of neurones excited by ACh during barbiturate anaesthesia has been reported for several areas of the brain (von Baumgarten *et al.*, 1963 ; Randić *et al.*, 1964 ; Salmoiraghi & Stefanis, 1965), although others have found that barbiturate anaesthesia did not affect the sensitivity of neurones to ACh (Andersen & Curtis, 1964 ; Crawford & Curtis, 1966).

The brain stem has been proposed as the primary site at which the barbiturates produce their depressant effects (Killam, 1962) and an action in this region has been considered to be the mechanism for the production of anaesthesia (French *et al.*, 1953). In view of the marked reduction in the number of neurones excited by ACh in the brain stem of pentobarbitone-anaesthetized animals, a more extensive study with both microiontophoretically and intravenously administered pentobarbitone was undertaken in order to explore this relationship more fully.

Microiontophoretic application of pentobarbitone blocked the excitant effects of ACh on most of the neurones studied. This antagonism was specific even though its demonstration was occasionally complicated by a non-specific depression

of the spontaneous neuronal firing rate. A similar selective antagonism of ACh excitation was present after i.v. administration of pentobarbitone or thiopentone. Studies on synaptic transmission in the cuneate nucleus have indicated that pentobarbitone had a specific depressant effect on postsynaptic excitation (Galindo, 1969) and selective depression of ACh excitation after i.v. pentobarbitone has been demonstrated in cortical neurones (Krnjević & Phillis, 1963), thalamic neurones (McCance *et al.*, 1968) and in the caudate nucleus (Bloom *et al.*, 1965). However, non-specific depression of ACh excitation has also been reported in cortical neurones (Crawford & Curtis, 1966, Crawford, 1970) after microiontophoretic application of pentobarbitone and it is possible that non-specific effects of pentobarbitone when applied at higher concentrations than in the present study may have masked any selective actions.

One of the characteristic effects of pentobarbitone, applied either iontophoretically or administered i.v., was the depression of spontaneous neuronal firing rate. Depression of spontaneous neuronal firing by pentobarbitone after microiontophoretic application (Bradley & Wolstencroft, 1965; Crawford & Curtis, 1966; Galindo, 1969; Crawford, 1970) or i.v. administration (Gauthier, Mollica & Moruzzi, 1956; Schlag, 1956; Yamamoto & Schaeppi, 1961; Rosina & Sotgiu, 1967) has been reported and may be due to depression of the release of ACh (Paton & Speden, 1965; Weakly, 1969).

The evidence for a selective blocking action of ACh excitation by pentobarbitone, which has been reported here and in previous studies, suggests that depression of neuronal firing rate may be due to an antagonism by pentobarbitone of endogenously released ACh, the latter contributing to the maintenance of neuronal tone. On the other hand, it could be argued that the effects seen with pentobarbitone might be due to a number of contributory factors which may include the ones mentioned above. A significantly greater proportion of slower firing neurones was observed in pentobarbitone-anaesthetized animals and this could be due to antagonism of endogenous ACh. It is interesting that the firing frequencies of neurones in the hypothalamus of urethane-anaesthetized rats (Cross & Dyer, 1971) and in the brain stem of urethane- or tribromoethanol-anaesthetized animals were not significantly different from those in controls. The fact that neuronal responses to postulated transmitter substances under these conditions were not modified in the same way as in pentobarbitone anaesthesia makes the site and mode of action of these anaesthetic agents more difficult to explain.

It has been suggested that pentobarbitone occupies ACh receptors in the postsynaptic membrane (Sato, Austin & Yai, 1967; Galindo, 1969) and that pentobarbitone blocks neuromuscular transmission by rendering the end plate receptors unreactive to the depolarizing effects of iontophoretically applied ACh (Thesleff, 1956). These results however, must be considered in the light of evidence from studies in the frog neuromuscular junction (Adams, Cash & Quilliam, 1970) suggesting that intrinsic and extrinsic ACh may act on different groups of receptors. The results presented here support the theory that pentobarbitone acts on postsynaptic sites and affects neurotransmission by interfering with the ability of ACh to produce depolarization.

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