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Drugs and PGO Waves in the Lateral Geniculate Body of the Curarized Cat
II. PGO wave activity and brain 5-hydroxytryptamine

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Abstract—Ponto-geniculo-occipital (PGO) waves induced either by the benzoquinolizine derivative, Ro 4-1284 (= PGO_{1284}), or by the inhibitor of tryptophan hydroxylase, *p*-chlorophenylalanine (= PGO_{PCPA}), were continuously recorded and counted in the lateral geniculate bodies of unanaesthetized immobilized cats as described in the preceding communication. The effect of various drugs interacting with central 5-hydroxytryptaminergic mechanisms on the density (number hr^{-1} or number 0.5 hr^{-1}) of PGO waves was investigated.

The density of PGO waves was dose-dependently decreased and the waves were eventually abolished by the precursors of 5-hydroxytryptamine (5-HT), L-tryptophan and 5-hydroxytryptophan. 5-HT itself, injected intracerebroventricularly, tended to diminish the density. Lysergic acid diethylamide (LSD) was an extremely potent depressor of PGO_{1284} and PGO_{PCPA}; psilocybine was almost as potent as LSD, whereas higher doses of N,N-dimethyltryptamine, bufotenine and yohimbine were required for the reduction of PGO waves. A number of tricyclic antidepressants reduced the density of PGO waves, their order of potency corresponding to that reported for their inhibitory effect on the uptake of 5-HT; tertiary amines were more potent than secondary amines. Desipramine was more potent on PGO_{PCPA} than on PGO_{1284}. Two dibenzothiepine antipsychotics, methiothepin and octoclothepin, increased the density of PGO_{1284} and PGO_{PCPA} and also induced PGO waves in untreated cats; the evidence suggests that these two compounds antagonize the effect of endogenous 5-HT in the brain.

These results confirm some earlier findings and augment the pharmacological pieces of evidence for the strong inhibitory influence of 5-HT neurones on the generation of PGO waves.

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Introduction

In the preceding paper (43) we have described two experimental situations in which PGO waves can be obtained in a reproducible manner over a period of several hr in the curarized cat. A fairly regular continuous PGO wave activity occurs shortly after the i.p. injection of Ro 4-1284, a benzoquinolizine derivative which impairs the storage capacity of neurones for monoamines (39). The compound very markedly reduced the brain level of noradrenaline (NA) at the dose used whereas the 5-hydroxytryptamine (5-HT) content declines only to about 60 % (40, 43). Functionally, the cats injected with Ro 4-1284 may be considered to have a virtually eliminated central noradrenergic synaptic transmission and a slightly reduced 5-hydroxytryptaminergic transmission. On the other hand, pretreatment of cats with *p*-chlorophenylalanine (PCPA) on the 3 days before the acute experiment induced an intermittent PGO wave activity, characterized by periodic bursts of activity with silent intervals of variable duration. The 5-HT content was very markedly and the NA content moderately decreased. Functionally, the 5-HT system seems to be greatly reduced in these cats, whereas the NA system appears to be little affected. Procedures have been elaborated that allow a quantitative evaluation of the effects of drugs on the PGO waves in the lateral geniculate bodies (LGB) induced by either Ro 4-1284 or PCPA (termed PGO_{1284} and PGO_{PCPA}).

Impairment of 5-hydroxytryptaminergic transmission, either by lesions of the raphé nuclei containing 5-HT neurones (8, 28, 48), or by chemical lesions of 5-HT neurones by 5,6-dihydroxytryptamine (16) or by the inhibition of 5-HT synthesis with PCPA (11, 29) induced the appearance of PGO wave activity in the cat. Jouvet (26) and Brooks and Gershon (5, 6) and Dement (13) postulated that the 5-HT neurones acted as an inhibitory system in the modulation of PGO wave activity.

This view receives further support from the pharmacological studies presented below.

Methods

Data were collected from approximately 160 cats. The preparation of the animals for the acute experiment, the induction of PGO wave activity by Ro 4-1284 and by PCPA and the technique for recording and counting PGO waves in the LGB have been described in detail in a preceding paper (43).

List of abbreviations: 5-HT = 5-hydroxytryptamine. — LGB = lateral geniculate body. — NA = noradrenaline. — PGO waves = ponto-geniculo-occipital waves. — PGO_{1284} = PGO-waves induced by Ro 4-1284. — PGO_{PCPA} = PGO-waves induced by PCPA. — PCPA = *p*-chlorophenylalanine. — REM = rapid eye movement. — Ro 4-1284 = 2-hydroxy-2-ethyl-3-isobutyl-9, 10-dimethoxy-1, 2, 3, 4, 6, 7-hexahydro-11bH-benzo(*a*)quinolizine hydrochloride.

The evaluation of the effects of drugs on PGO_{1284} and PGO_{PCPA} was performed in the following way (see Fig. 1):

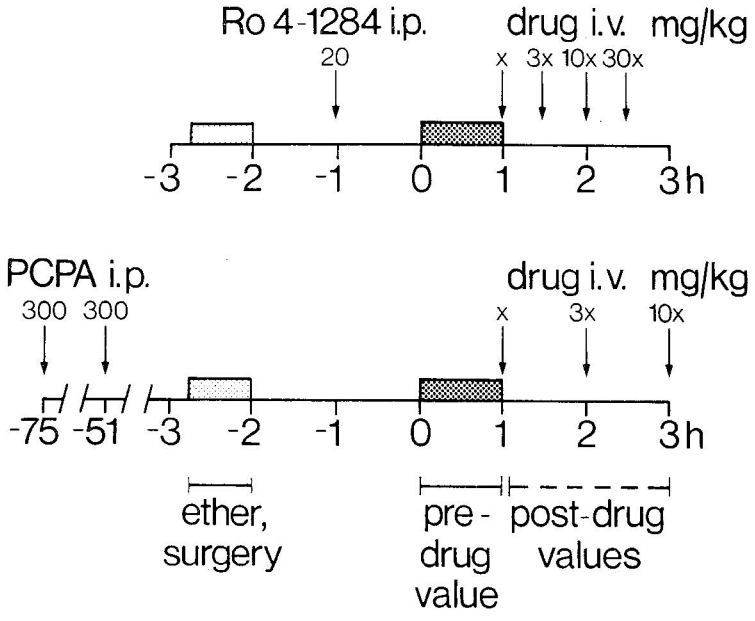

FIG. 1

Schematic presentation of the experimental protocol used for the study of the effects of drugs on PGO_{1284} and on PGO_{PCPA}. At time zero the counting of PGO waves started.

a) *PGO_{1284}:* The density of PGO_{1284} counted during the 2nd hr following the injection of Ro 4-1284 was taken as the "pre-drug control value". Thereafter the compounds to be tested were injected i.v. starting at the smallest dose. Increasing doses were given at intervals of 0.5 hr.

b) *PGO_{PCPA}:* The density of PGO_{PCPA} during the 74th hr following the first of 2 injections of PCPA was taken as the "pre-drug control value". In a few instances, where positioning of the electrodes for optimal recording of geniculate PGO waves took more time, the 75th hr served as the control period. Compounds to be tested were then injected i.v. in incremental doses; the interval between two injections was 1 hr, in contrast to the experiments with Ro 4-1284, because of the irregular occurrence of bursts of PGO_{PCPA} (43). The 6 min immediately following the injection of drugs were not used for the calculations in order to eliminate non-specific acute effects of the injections. In each cat the density of PGO waves during the 30 min (for PGO_{1284}) or the hr (for PGO_{PCPA}) following the i.v. injection of a given dose of a compound was expressed in per cent of the pre-drug value of the same animal. For every dose the mean percentage change

(± its S.E.) of the density in at least 3 animals was calculated. These means were then compared with the density of PGO waves during the corresponding time in Ro 4-1284- and PCPA-treated animals given saline instead of the compounds, since the density of PGO waves slowly declined following the pre-drug control period (43). The effect of a given dose was eventually expressed in per cent of the density in saline-injected animals. The final figures for a certain drug effect therefore contain both an intraindividual (post-drug to pre-drug values) and an interindividual comparison (mean post-drug to mean post-saline in a control group). For statistical comparisons Student's "*t*"-test was used. For intraventricular injections of drugs a cannula was stereotaxically implanted in the left lateral ventricle to allow the repetitive administration of saline or of test substances. Each dose was injected slowly in 0.1 ml kg^{-1} solution for the first 5 doses and 0.3 ml kg^{-1} for the last one. The results are reported using as controls animals injected intracerebroventricularly with saline. These animals showed no consistent difference from untreated animals; only the individual variations around the mean were greater.

The *drugs* used were: amitriptyline hydrochloride, 2-bromolysergic acid diethylamide tartrate (BOL 148), bufotenine oxalate, butriptyline, chlordesipramine hydrochloride, chlorimipramine hydrochloride, cinanserin (Squibb), cocaine HCl, cyproheptadine hydrochloride, desipramine hydrochloride, dibenzepine hydrochloride, N,N-dimethyl-tryptamine-oxalate (DMT), doxepine

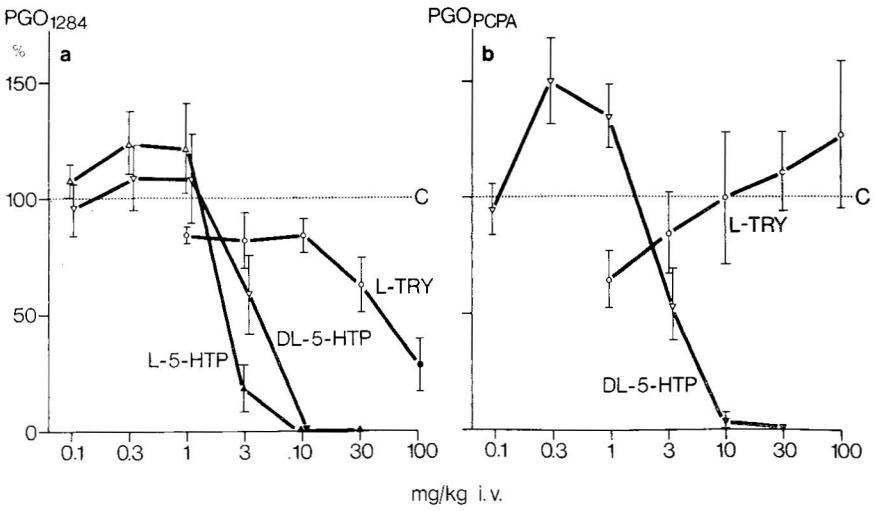

FIG. 2

The effects of L-tryptophan (L-TRY), 5-hydroxy-L-tryptophan (L-5-HTP) and of 5-hydroxy-DL-tryptophan (DL-5-HTP) on the density of PGO_{1284} (*a*) and of PGO_{PCPA} (*b*). The points are the mean of at least 3 animals, the vertical bars indicate their S.E.M. The pre-drug control values are taken as 100 %; the effects of drugs are corrected for the progressive decline of PGO waves in control animals (see Methods). Statistically significant differences ($p < 0.05$) from the control value are indicated by solid symbols.

hydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), D,L-5-hydroxytryptophan (DL-5-HTP), L-5 hydroxytryptophan (L-5-HTP), imipramine hydrochloride, iprindole, ketipramine maleate, lysergic acid diethylamide-D-tartrate (LSD), maprotiline methanesulphonate, melitracene hydrochloride, methiothepin maleate, methysergide dimaleate, mianserin hydrochloride, nortriptyline hydrochloride, noxiptyline, octoclothepin methanesulfonate, opipramol dihydrochloride, *p*-chlorophenylalanine (PCPA), prothiadene hydrochloride, protriptyline hydrochloride, psilocybine, quipazine maleate (2-(1-piperazinyl) quinoline maleate) (Miles Lab.), Ro 4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b-benzo(a) quinolizine hydrochloride), trimipramine maleate, tryptamine hydrochloride, L-tryptophan and yohimbine.

Results

1) *Precursors of 5-HT*

L-Tryptophan and 5-HTP depressed and eventually abolished PGO_{1284} in a

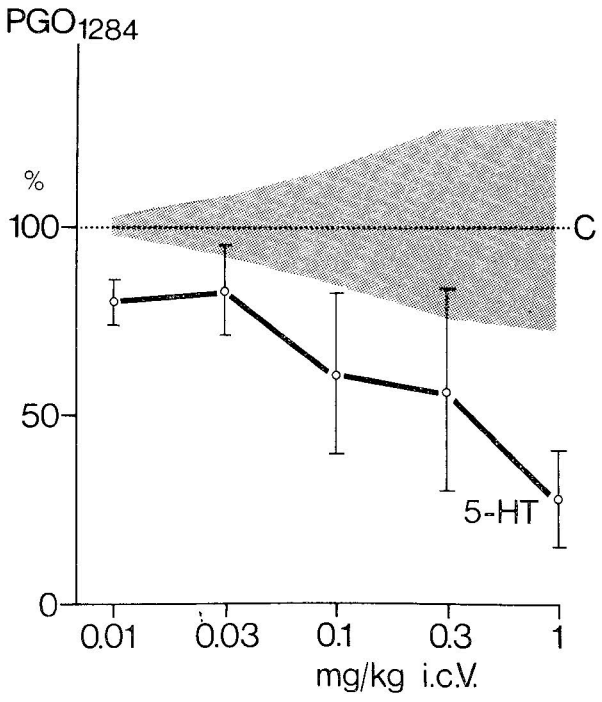

FIG. 3

Effect of 5-HT, injected into the lateral ventricle, on the density of PGO_{1284} ($n = 4$). The shaded area indicates the range of variation observed in animals injected with saline only.

dose-dependent manner (Fig. 2). The ED_{50} of L-5-HTP was approximately 2 mg kg^{-1} i.v., that of DL-5-HTP 5 to 6 mg kg^{-1}. L-Tryptophan had 1/25 the potency of L-5-HTP. The effects of DL-5-HTP were the same in PCPA-treated and in cats given Ro 4-1284. However, tryptophan was inactive up to 100 mg kg^{-1} i.v. on PGO_{PCPA}.

2) *5-HT intracerebroventricularly*

5-HT tended to decrease the PGO_{1284} density when administered into the lateral ventricle, although this decrease was not significantly different from that in controls injected with the corresponding quantities of saline (Fig. 3). In one cat, 5-HT was administered i.v. in doses up to 100 mg kg^{-1}; it had no effect on PGO_{1284}.

3) *Tryptamine*

Cumulative doses of tryptamine up to 30 mg kg^{-1} i.v. had no statistically

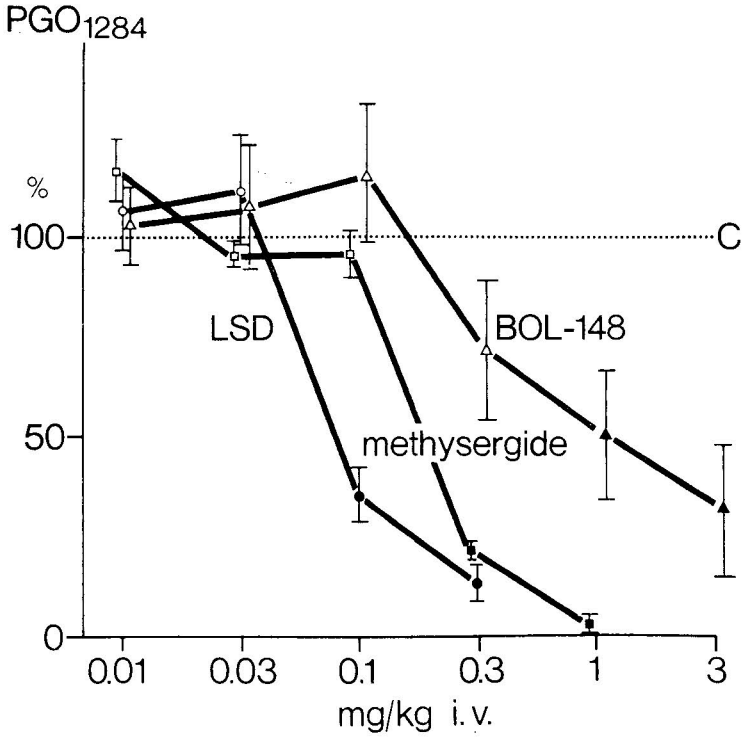

FIG. 4

The effects of LSD, BOL-148 and methysergide on the density of PGO_{1284}. Evaluation and presentation as in Fig. 2. The points are the means of at least 3 experiments.

significant effect on the PGO_{1284}, although the highest dose tended to reduce the wave density to 50 % ($n = 3$).

4) *LSD and related compounds*

LSD was a very potent depressant of PGO_{1284} with an ED_{50} of approximately 50 $\mu g\ kg^{-1}$ i.v. (Fig. 4). Similar effects were observed with methysergide which was only half or 1/3 as potent as LSD (Fig. 4). One mg kg^{-1} of methysergide i.v. abolished the PGO_{1284}. The bromo analogue of LSD (BOL-148) had 1/10 the potency of LSD (Fig. 4).

In PCPA-treated animals, LSD was at least as potent a depressant of PGO waves as in Ro 4-1284 cats; a single dose of 0.1 mg kg^{-1} LSD i.v. suppressed PGO_{PCPA} for at least 3 hr ($n = 3$).

5) *Other indolealkylamines*

Psilocybin was at least as potent as LSD in reducing the density of PGO_{1284}

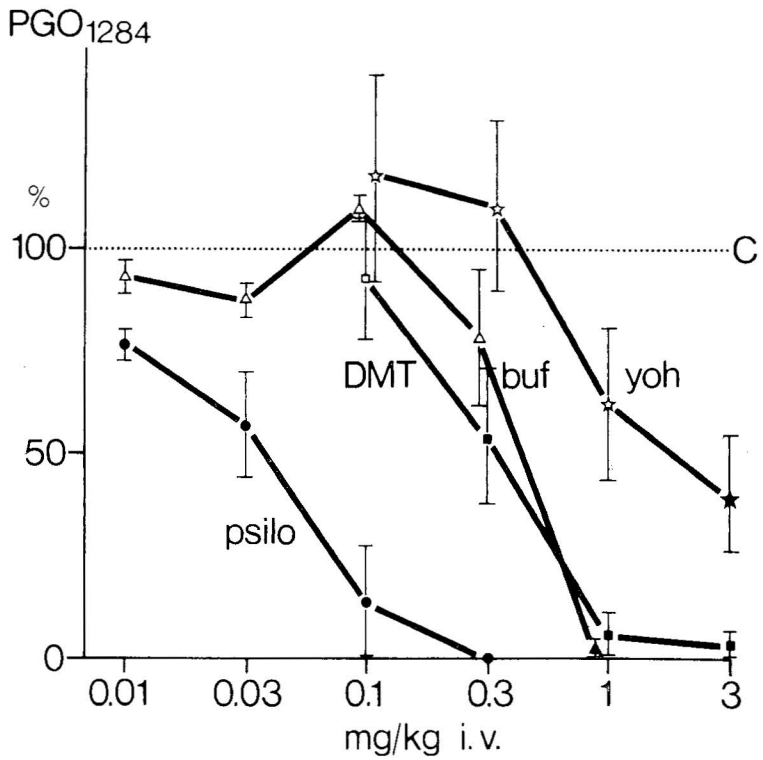

FIG. 5

The effects of psilocybin, N,N-dimethyltryptamine (DMT), bufotenin, and yohimbine on the density of PGO_{1284}. Each point is the mean of at least 3 experiments. Evaluation and presentation as in Fig. 2.

(Fig. 5), as after 0.3 mg kg^{-1} the PGO-waves were abolished. *N,N-dimethyltryptamine (DMT)* and *bufotenine* had approximately 1/10 the potency of psilocybine (Fig. 5). *Yohimbine* was considerably less potent and a dose of 3 mg kg^{-1} was required to reduce the density of PGO_{1284} to 50 % (Fig. 5).

6) *Tricyclic antidepressants*

The main mechanism of action of tricyclic antidepressants, according to present views, is the inhibition of the neuronal re-uptake of NA and 5-HT into their respective neurones. Some of these compounds act predominantly on either NA or 5-HT uptake, while others have similar activities on both types of uptake. Changing only the substitution on the side-chain nitrogen markedly alters the relative potencies of tricyclic amines as inhibitors of either amine. Imipramine and its secondary amine derivative, desmethylimipramine, are a typical example and were therefore studied in some detail.

Fig. 6 shows that desmethylimipramine had only about 1/10 the potency of imipramine as a depressant of PGO_{1284}. Imipramine was very potent, the ED_{50} being approximately 0.2 mg kg^{-1} i.v., and abolition of PGO_{1284} occurring between 1 and 3 mg kg^{-1}. No difference, however, was found between the potencies of the tertiary and secondary amine for the depression of PGO_{PCPA}.

A series of antidepressants was studied in cats given Ro 4-1284. Fig. 7 shows their relative potencies as depressants of PGO_{1284}. Indicated are the ED_{50}'s. The

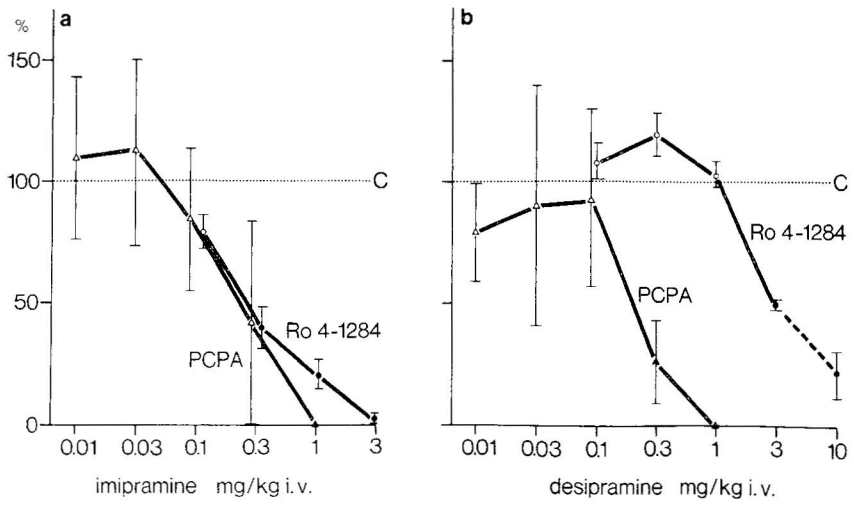

FIG. 6

The effects of imipramine (left) and desipramine (right) on the density of PGO_{1284} and PGO_{PCPA}. Evaluation and presentation as in Fig. 2. Each point is the mean of at least 3 experiments. The dotted line on the right-hand graph indicates the dose range in which the basal activity of the lateral geniculate body was grossly altered.

most potent compound was chlorimipramine (ED_{50} 60 μg kg^{-1} i.v.), followed by imipramine, amitriptyline, protriptyline, chlordesipramine and others. Iprindole, which has no inhibitory effect on the uptake of monoamines, was inactive up to 300 times the ED_{50} of chlorimipramine. Marked differences between tertiary and secondary amines were found not only with imipramine and desimipramine but also with amitriptyline and nortriptyline and with chlorimipramine and chlordesipramine. Maprotiline, reported not to affect the uptake of 5-HT (32), was inactive on PGO_{1284} up to 10 mg kg^{-1}, but very potently depressed PGO_{PCPA} (ED_{50} 0.6 mg kg^{-1}).

Cocaine, another inhibitor of both 5-HT and NA uptake, depressed PGO_{1284} with an ED_{50} of about 0.5 mg kg^{-1}.

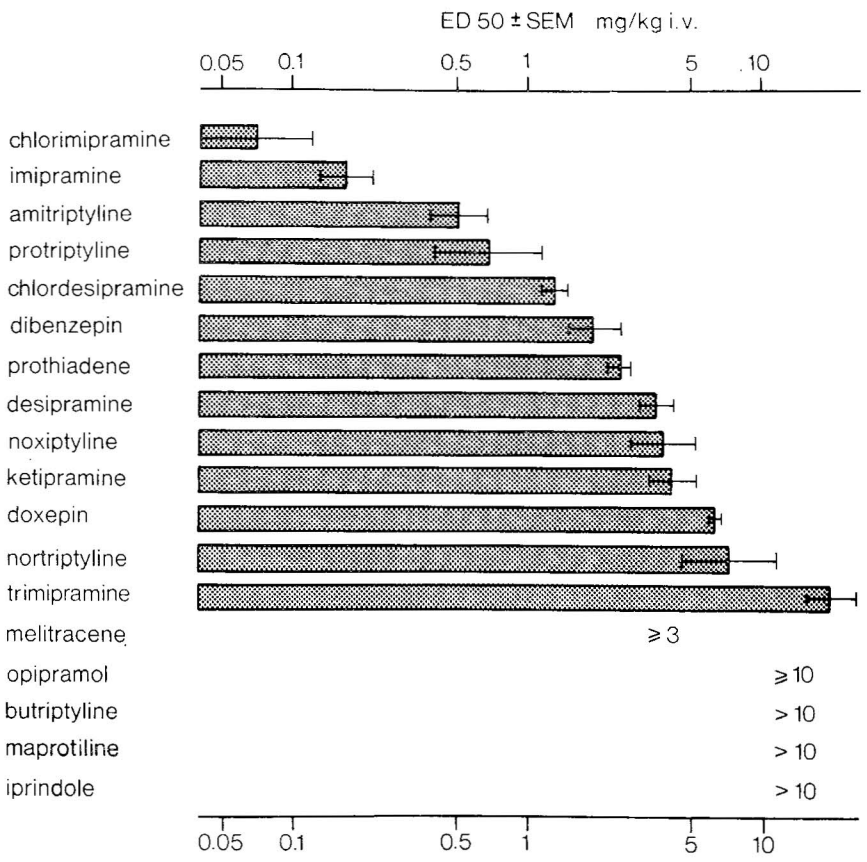

FIG. 7

Comparison of the depressant effect of antidepressants on the density of PGO_{1284}. Indicated are the mean ED_{50} and S.E.M. from at least 3 experiments, i.e. the doses that reduced the number of PGO_{1284} to half the value counted at the corresponding time in control animals (see Methods).

7) *Quipazine*

Quipazine depressed PGO_{1284} in 3 cats, the ED_{50} being 2.5 mg kg^{-1}.

8) *Central 5-HT antagonists*

Of the 3 compounds claimed to be antagonists of central actions of 5-HT, cinanserin proved inactive up to 10 mg kg^{-1} i.v. (Fig. 8). Mianserin, a strong antagonist of peripheral 5-HT effects, increased significantly the density of PGO_{1284} in doses of 0.3, 1 and 3 mg kg^{-1}; the maximal increase of PGO_{1284} was about 70 % (Fig. 8). Cyproheptadine slightly increased PGO_{1284} at the dose of 1 mg kg^{-1} but depressed them with 10 mg kg^{-1} (Fig. 8).

The 2 most potent compounds which also produced the most marked increase of PGO_{1284} were methiothepin and octoclothepin, two novel dibenzothiepin neuroleptics (Fig. 8). Methiothepin had a similar activity on PGO_{1284} and PGO_{PCPA}. The effects in PCPA-treated animals showed great variations, owing to the marked inter-individual differences of pre-drug values.

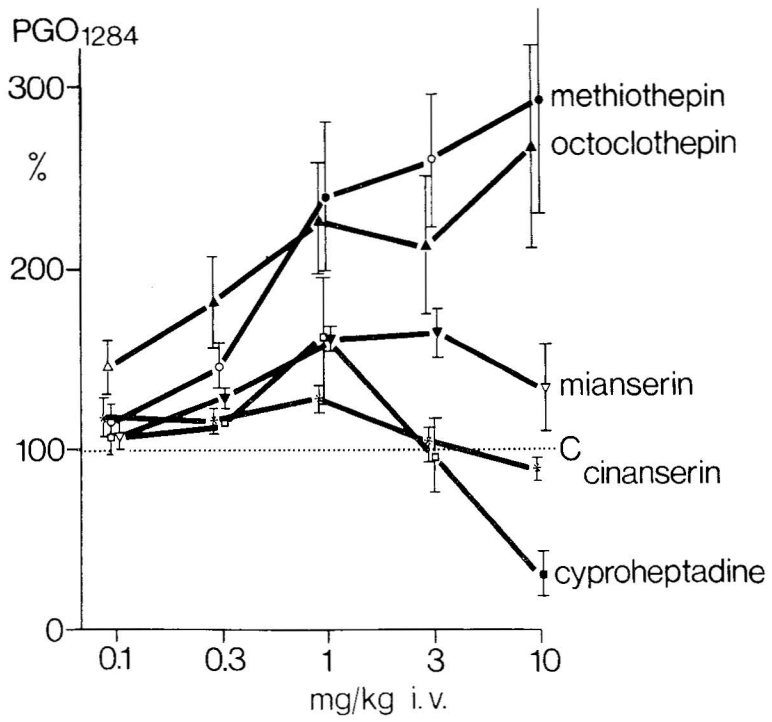

FIG. 8

Effects of methiothepin, octoclothepin, mianserin, cinanserin and cyproheptadine on the density of PGO_{1284}. Evaluation and presentation as in Fig. 2. Each point is the mean of at least 3 experiments.

Methiothepin antagonized the depressant effect of 5-HTP on PGO_{1284} (Fig. 9) and of LSD on PGO_{PCPA}. The dose-response curve for the depressant effect of 5-HTP was shifted to the right by a factor of 5 after 10 mg kg^{-1} methiothepin i.v.

When methiothepin 10 mg kg^{-1} i.v. was injected into cats that had received neither Ro 4-1284 nor PCPA, we observed the immediate onset of a continuous discharge of PGO waves lasting several hr ($n = 4$). The density of the PGO waves was maximal (200 to 350 hr^{-1}) during the 1st hr after the injection. In one cat the waves occurred in bursts.

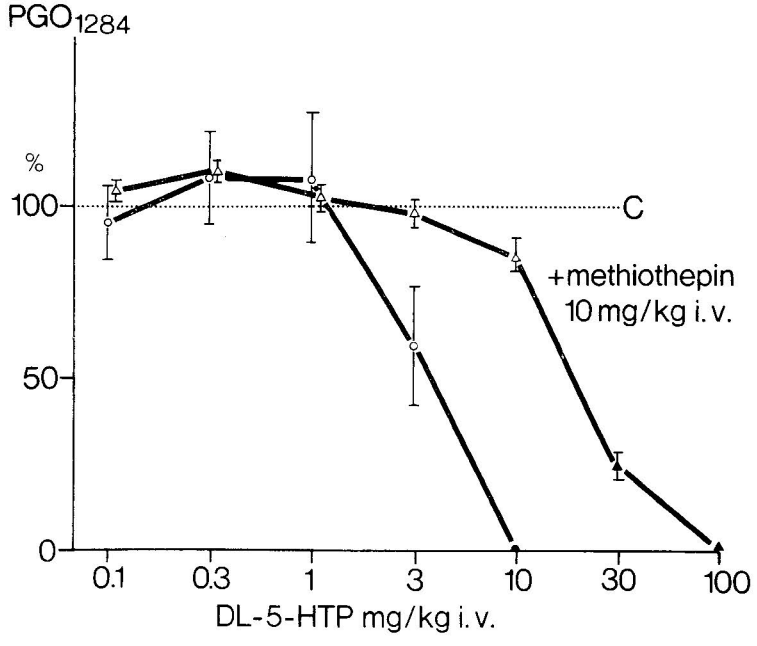

FIG. 9

Antagonism by methiothepin of the depressant effect of 5-HTP on the density of PGO_{1284}. Evaluation and presentation as in Fig. 2. Each point is the mean of at least 3 experiments.

9) *PGO waves in the absence of drugs*

In a few cats (8 out of about 3000 studied so far) PGO wave activity in the LGB was present before Ro 4-1284 was injected. These animals were kept under the usual experimental conditions without being injected with any drug and after several hr of recording the 5-HT content of their brains was determined. The amine level was lower in these cats than in unselected control animals. Since the animals had not been carefully observed before the experiment, we do not know whether it would have been possible to select cats with spontaneous PGO wave

activity on the basis of special behavioural parameters. The reason for the lowered brain 5-HT levels are unknown.

Discussion

Our results support the view proposed by Jouvet (26, 27) and Brooks and Gershon (5–7) that the activity of 5-HT neurones inhibits the generation of PGO waves; previous findings and the present results clearly indicate that the presence or absence of PGO waves under various conditions depends primarily on reduced or enhanced synaptic concentrations of 5-HT, respectively. The arguments in favour of the modulating role of 5-HT are derived, on the one hand, from the appearance of PGO waves after physical (coagulation) and chemical (i.c.v. injections of 5,6-dihydroxytryptamine) lesions of 5-HT neurones, after pharmacological reduction of the 5-HT available for synaptic transmission (Ro 4-1284, PCPA) and after blockade of 5-HT receptors, and, on the other hand, from the suppression of PGO_{1284} and PGO_{PCPA} by precursors of 5-HT, by agonists of 5-HT receptors and by agents which increase the synaptic concentration of 5-HT by blocking its neuronal re-uptake.

The PGO waves induced by Ro 4-1284 were rapidly depressed and eventually abolished by low doses of *5-HTP*. It has previously been reported that 5-HTP abolishes REM sleep and the corresponding PGO waves (10) as well as the PGO waves induced by reserpine (12) or by PCPA (26). *Tryptophan* was considerably less potent. Whereas 5-HTP can be decarboxylated to 5-HT intraneuronally in 5-hydroxytryptaminergic and catecholaminergic neurones as well as extraneuronally (e.g. in the capillaries), the formation of 5-HT from tryptophan is likely to occur only in 5-hydroxytryptaminergic neurones. The depression of PGO_{1284} by tryptophan is therefore very probably due to the formation and subsequent release of 5-HT at those synapses where 5-HT acts physiologically as an inhibitor of PGO wave generation. 5-HTP depressed the PGO waves induced by Ro 4-1284 and PCPA to the same degree, tryptophan however was inactive on PGO_{PCPA}. Since PCPA inhibits tryptophan hydroxylase, the inactivity of tryptophan is not unexpected. *Tryptamine*, if formed at all from tryptophan, does not seem to be able to mimic the action of 5-HT. The inactivity of tryptamine injected i.v. points in the same direction, though rapid enzymatic destruction might be responsible. In fact, after pretreatment with the monoamine oxidase, pargyline, tryptamine abolished PGO waves induced by reserpine (Depoortere, personal communication).

LSD was the most potent agent found so far to depress PGO waves induced by Ro 4-1284 as well as by PCPA. Our findings on PGO_{1284}, reported earlier (23), were extended to PGO waves induced by reserpine (15). This effect adds further strong evidence for the view that LSD mimics at least part of the central effects of 5-HT. A reduction by LSD of the density of PGO_{PCPA} has also been observed in unrestrained cats (22); the induction by LSD of monophasic waves in the

lateral geniculate bodies of unrestrained and otherwise untreated cats (35, 49) might be eye movement potentials rather than PGO waves (22). The evidence speaks in favour of a direct action of LSD on 5-HT receptors, although identical effects of LSD on PGO_{1284} and PGO_{PCPA} are not a definitive proof for this assumption. Evidence for a 5-HT receptor stimulating effect of LSD is also provided by biochemical (3, 21) and neurophysiological (1, 2) data. Based on the finding that LSD by local application was more potent in depressing 5-hydroxytryptaminergic neurones of the raphé nuclei than neurones receiving an inhibitory input from the latter (2), one should expect that the hallucinogen increased the density of PGO_{1284}, at least in low doses. This, however, was not observed in our experiments and the question, therefore, remains open whether the neurones involved in the generation of PGO waves are more sensitive to LSD than other cells with 5-hydroxytryptaminergic innervation. *Methysergide*, which like LSD is an antagonist of 5-HT at peripheral non-neuronal cells, had 1/3 the potency of LSD. This finding puts methysergide in the class of agonists at central 5-HT receptors. Two problems then arise. The first concerns the possible involvement of 5-HT receptor activation in the hallucinogenic action of LSD. Methysergide is a very weak hallucinogen, if any. Unless the penetration of methysergide were much less in man than in cats, our findings make a causal relationship between 5-HT receptor activation and hallucinogenic activity questionable. Perhaps the recently observed dopamine receptor activating property of LSD (38) is more relevant for the hallucinogenic activity. Our findings of a similar 5-HT-like action of LSD and methysergide are in conflict with those of Anderson (4), who found that both compounds were able to antagonize the effects of 5-HTP on the cat spinal cord. The possibility has to be considered that different types of 5-HT receptors exist in the CNS; this does not seem too remote a possibility in view of the presence of at least two different 5-HT receptors in the cells of the sympathetic superior cervical ganglion (20). LSD and methysergide have been reported earlier to suppress the PGO waves induced by both reserpine and PCPA (15). Different results with LSD have been obtained in unrestrained and undrugged cats: while it suppressed the PGO waves of REM phases of sleep, it enhanced the occurrence of PGO waves during the non-REM phases (49). The bromo analogue of LSD, *BOL-148*, believed to have little if any hallucinogenic activity, had only 1/10 the potency of LSD in depressing PGO_{1284}. Since *5-HT* itself virtually does not pass through the blood-brain barrier, it was injected into the lateral ventricle in the hope of obtaining LSD- or 5-HTP-like effects at least in high doses. No convincing effect of 5-HT was observed up to 1 mg intracerebroventricularly. However, this negative finding has little weight since monoamines injected into the cerebrospinal fluid only reach structures in the close vicinity of the inner surface of the brain. In the rabbit, intraventricular injections of small amounts of 5-HT suppressed monophasic waves in the medial trapezoid nucleus induced by reserpine (51).

Quipazine has been postulated to have central 5-HT-like actions (14, 18, 19, 42) and to block the uptake of 5-HT into striatal tissue *in vitro* (33), besides

possessing some actions in common with tricyclic antidepressants (41). Our finding that quipazine in rather high doses reduced the density of PGO_{1284} is in accordance with an action on 5-HT receptors or on 5-HT re-uptake, but does not prove it.

The depressant effect of *yohimbine* on PGO_{1284} cannot be explained with certainty. The molecular structure of the compound would be compatible with a 5-HT-like action for which some pharmacological evidence exists (36, 46, 47). However, yohimbine might also act on the re-uptake of 5-HT (25) or influence monoamines in still another way.

Reduction and abolition of PGO waves were obtained with a large number of tricyclic amines used as *antidepressants*. A main effect of these drugs seems to be the inhibition of neuronal uptake of monoamines. The compounds differ considerably in their selectivity for either NA or 5-HT uptake (9). For example, secondary tricyclic amines predominantly inhibit the uptake of NA, whereas tertiary derivatives are especially active on the uptake of 5-HT; maprotiline is a selective inhibitor of the uptake of NA (32). The order of potency of antidepressants for reduction of PGO_{1284} is surprisingly similar to that for the inhibition of 5-HT uptake as measured by chemical methods. The correlation between effects on PGO_{1284} and on chemically determined inhibition of 5-HT uptake has been worked out in detail (25). This study, on the one hand, underlines the usefulness of neuropharmacological and neurochemical methods for the evaluation of inhibitors of 5-HT uptake and, on the other hand, stresses further the determinant effect of endogenous 5-HT in cats treated with Ro 4-1284. The different noradrenergic and 5-hydroxytryptaminergic tone in animals treated either with Ro 4-1284 or PCPA is also convincingly demonstrated by the comparison of the effects of imipramine and desimipramine on PGO_{1284} and PGO_{PCPA}. Desimipramine which predominantly inhibits the uptake of NA (9) was distinctly less potent on PGO_{1284} than imipramine which predominantly inhibits the uptake of 5-HT (9). Moreover, desimipramine abolished PGO_{PCPA} with 1/10 the dose required for the same effect on PGO_{1284}. Maprotiline was even more selective as a depressant of PGO_{PCPA}. This confirms the view that in cats treated with Ro 4-1284 the noradrenergic system was virtually eliminated and the activity of the 5-hydroxytryptaminergic system only partially reduced, whereas the animals treated with PCPA had a markedly reduced activity of their 5-HT system and a nearly normal or fully functioning NA-system. Our findings with tricyclic antidepressants have an analogy with clinical data reporting a rapid suppression of REM sleep by these drugs; chlorimipramine was found to be the most potent, whereas trimipramine and iprindole were inactive (37).

If it is true that 5-HT has a decisive inhibitory action on the generation of PGO waves and if Ro 4-1284 predominantly depresses the NA system, antagonists of 5-HT would be expected to increase further the density of PGO_{1284} by removing the remaining synaptic activity of 5-HT. Such compounds were in fact found. When testing a series of neuroleptics, some of which possess 5-HT-antagonistic actions in the periphery, two were found to increase the density of

PGO_{1284} and PGO_{PCPA} and to induce PGO waves in otherwise untreated animals, these being *methiothepin* and *octoclothepin*, two potent dibenzothiepine neuroleptics (thioridazine and clozapine will be discussed in the following paper, 44). Methiothepin antagonized the depressant effect of 5-HTP and of LSD on PGO_{1284} and PGO_{PCPA}. Biochemical findings support the view that the compound in fact acts as central 5-HT antagonist: it increased the content of 5-hydroxyindolacetic acid, a metabolite of 5-HT, without changing the level of 5-HT itself (31, 34). These findings also indicate that blockade of 5-HT receptors initiates a positive feedback mechanism that activates the 5-HT neurones so as to overcome the blockade by an increased liberation of 5-HT. Further evidence for the central 5-HT antagonistic action of methiothepin was found in young chicks where the compound even in μg doses prevented the sleep-inducing effect of 5-HT injected i.v. (24) and in the cat lateral geniculate body, where it depressed the inhibitory effect of microelectrophoretically applied 5-HT (52).

Central 5-HT antagonistic actions have been described for *mianserin* (53), *cyproheptadine* (50) and *cinanserin* (17). Only the first 2 compounds increased PGO_{1284}; however, the intensity of their effect was clearly inferior to that of methiothepin and octoclothepin and, moreover, was seen only in a narrow dose range. Since all 3 compounds prevented the sleep-inducing effect of 5-HT in young chicks (24), albeit in much higher doses than the dibenzothiepines, they might have 5-HT antagonistic activity also in the cat, but other central actions probably prevail in higher doses and tend to overcome their effect on 5-HT receptors. The present evidence for a weak anti-5-HT effect of mianserin is difficult to reconcile with a reduction of brain 5-HT turnover in rats (30).

The view that 5-HT neurones are of uppermost importance for the phenomenon of PGO waves is based on straightforward pharmacological evidence and little doubt can exist that impulse flow in 5-hydroxytryptaminergic neurones suppresses the generation of PGO waves, whereas a reduced activity in these neurones represents a "gating mechanism" (5, 6, 45) for PGO wave activity. Nevertheless, not every drug effect on PGO waves induced by Ro 4-1284 (or a similarly acting drug) or PCPA can be ascribed simply to an interaction with central 5-hydroxytryptaminergic mechanisms. In the following paper (44) we intend to show that noradrenergic neurones are also intimately involved in the control of PGO wave activity. The decision whether a given compound affects PGO waves through the interaction with 5-HT or NA mechanisms frequently requires data from other experiments.

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