die Mikrosomen der so rasch wie möglich entnommenen Lebern nach vorheriger Perfusion mit 0,9%ig. Kochsalzlösung gewonnen[6] und der mikrosomale Gehalt an Cytochrom P450 bestimmt[7].

Wie die Tabelle zeigt, nimmt die Konzentration von Cytochrom P450 bei Thioacetamid-vergifteten Tieren im Vergleich zu unvergifteten deutlich ab. Nach prophylaktischer Silymarinapplikation ist eine statistisch signifikante Normalisierung der verminderten Messgrösse nachweisbar ($2\alpha < 0.01$).

Die vorliegenden Untersuchungen berücksichtigen nicht, inwieweit das Lösungsmittel Polyvinylpyrrolidon den mikrosomalen Gehalt an Cytochrom P450 beeinflusst. Da jedoch alle untersuchten Gruppen in gleicher Weise mit Polyvinylpyrrolidon behandelt wurden, ist eine evtl. zusätzliche Wirkung dieser Substanz – der Cytochrom P450-Gehalt der Basisgruppe beträgt etwa $^2/_3$ der Norm – auf die untersuchte Messgrösse für die Versuchsaussage unerheblich. Auffälligerweise ist die Streuung der Messwerte des Thioacetamid-vergifteten Kontrollkollektivs im Vergleich zum Basiskollektiv sowie zu den mit Silymarin behandelten Versuchstieren deutlich geringer, ohne dass hierfür eine Erklärung gegeben werden kann. Bei Anwendung eines parameterfreien Signifikanztests wie des Rangsummentests nach Wilcoxon ist die unterschiedliche Varianz der untersuchten Gruppen jedoch ohne Einfluss auf die statistische Sicherung der Messergebnisse.

Die Wirkung von Silymarin-N-methylglucamin-Salz auf den mikrosomalen Gehalt von Cytochrom P450 der Rattenleber bei akuter Thioacetamidintoxikation (Mittelwerte ± Standardabweichungen)

	n*Mol* Cytochrom P450/ mg mikrosomales Protein
B	$0{,}42 \pm 0{,}13$
K	$0{,}25 \pm 0{,}01$ ↓
V	$0{,}37 \pm 0{,}10$ *

B, unvergiftetes Basiskollektiv, $n = 10$; K, Thioacetamid-vergiftetes Kontrollkollektiv, $n = 10$; V, Thioacetamid-vergiftetes, mit Silymarin-N-methylglucamin-Salz vorbehandeltes Versuchskollektiv, $n = 9$ (Einzelheiten s. Text). Statistisch signifikante Unterschiede im Rangsummentest nach Wilcoxon: Kontrollkollektiv-Basiskollektiv: ↓, $2\alpha < 0{,}01$; Versuchskollektiv-Kontrollkollektiv; *, $2\alpha < 0{,}01$.

Summary. 24 h after Thioacetamide application (300 mg/kg body weight) on rats, microsomale Cytochrome P450 content is diminished about 40%. Prophylactic i.p. application of 400 mg Silymarine-N-methylglucamine salt, dissolved in 4% Polyvinylpyrrolidone solution, given 1 h before application of Thioacetamide, counteracts this diminution.

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Dopamine Receptor Blockade by Neuroleptic Drugs in *Aplysia* Neurones

Dopamine receptor blockade has been suggested as an important mechanism of action of antipsychotic drugs[1]. The evidence for this mechanism includes the occurrence of 'parkinsonian' side effects in patients during therapy with neuroleptic drugs[2–5]. In animal experiments, neuroleptic drugs can reverse stereotyped behavior induced by apomorphine and amphetamine[6,7]. Phenothiazines and butyrophenones enhance dopamine metabolism, probably as a consequence of a blockade of dopamine receptors in the brain[8–14]. Such blockade has recently been demonstrated in vitro using dopamine-sensitive adenyl cyclase from rat corpus striatum[15,16] and from calf retina[17] as a receptor system. The blockade of dopamine receptors by antipsychotic drugs has also been suggested by their conformational similarities with dopamine[18].

Neurophysiological results are consistent with the interpretation that the mechanism of neuroleptic action is by dopamine receptor blockade, but direct evidence is scarce. Depression of excitatory responses in the caudate nucleus by haloperidol[19] and antagonism between the action of dopamine and chlorpromazine in striatal neurones have been obtained[20], but local anesthetic effects could not be ruled out completely. Chlorpromazine interferes with the apomorphine effect in nucleus solitarius neurones[21]. Antipsychotic phenothiazines and haloperidol inhibit the depression of the spontaneous firing rate of mesencephalic dopaminergic neurones

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caused by amphetamine[22]. These results provided indirect evidence for dopamine receptor blockade by a polysynaptic neuronal feedback mechanism[8] which was considered to be responsible for these observed changes in spontaneous activity of dopaminergic cells[22].

Receptors of putative synaptic transmitters may be studied directly in ganglion preparations of molluscs by intracellular recording of neuronal activity and by extracellular microelectrophoretic application of the test substances[23–29]. This preparation has been used successfully for studying pharmacological effects on the receptors[25–29]. We have applied this model for the investigation of the blocking action of antipsychotic drugs on dopamine receptors.

Methods. The experiments were performed on neurones of the intact isolated abdominal ganglion of the marine mollusc *Aplysia californica*. In order to preserve morphological orientation and to prevent losing the cells during changes of perfusate, the capsule of the ganglion was not dissected. The somata of identified neurones[24] were penetrated with microelectrodes and intracellular voltages were recorded using standard techniques. Anodal current pulses of 0.1 to 3.0 sec duration and 50 to 400 nA were used to iontophoretically eject dopamine (0.5 *M*), acetylcholine (1.0 *M*) or 5-hydroxytryptamine (0.05 *M*) from up to 3 independent microelectrodes positioned on or near the cell surface. The investigated drugs were added directly in concentrations of 10 μM to 100 μM to the artificial seawater perfusate (pH 7.8–8.0, 16 °C). Haloperidol and fluphenazine were chosen as neuroleptic agents, and mepazine was used as phenothiazine with poor antipsychotic effect[1]. Each putative neurotransmitter substance may hyperpolarize (H) or depolarize (D) the membrane depending upon the type of cell. The different responses appear to involve a specific increase in the membrane permeability for different ions[25, 26, 28]. The rapid desensitization of dopamine and 5-hydroxytryptamine receptors[26, 27] necessitated the use of intervals of 2 to 5 min between successive applications of these agents.

Results. Haloperidol, added to the perfusate in a concentration of 50 μM, decreased or blocked in 13 neurones (type R4–R12, RB, RC) H-response and in 3 neurones (type L7) D-response elicited by dopamine (Figure 1a). Fluphenazine was tested in concentrations of 10 and 100 μM in 6 neurones (type RB(2), L7(2), R6 and LC). In 4 of these neurones, 10 μM fluphenazine caused a decrease or blockade of the dopamine response (Figure 1b). In all 6 cells responses to the test stimuli of dopamine were blocked by 100 μM fluphenazine, but small responses could be elicited in most cases by electrophoretic stimuli of longer duration and higher intensity, suggesting a competitive mechanism. Both D- and H-response to dopamine were affected similarly by the drugs. Because of differences between experiments caused by interindividual variation in structure of the tissue covering the investigated cells and the different positions of electrophoresis microelectrodes, a quantitative comparison was not attempted. Depending on the thickness and toughness of the capsule, which delayed the permeation of the drugs, the effects on the response to dopamine were observed between 8 and 40 min after starting the perfusion with seawater containing the neuroleptic drugs. Washing with drug-free seawater for 30–100 min reversed the effects of the neuroleptic drugs on the dopamine response in all neurones. In contrast to these observations, responses to microelectrophoretic application of acetylcholine or 5-hydroxytryptamine were not impaired by perfusion with haloperidol or fluphenazine (Figure 2).

The non-antipsychotic phenothiazine mepazine was tested in 2 neurones (R6 and LC) in which dopamine response was severely depressed by 10 μM fluphenazine

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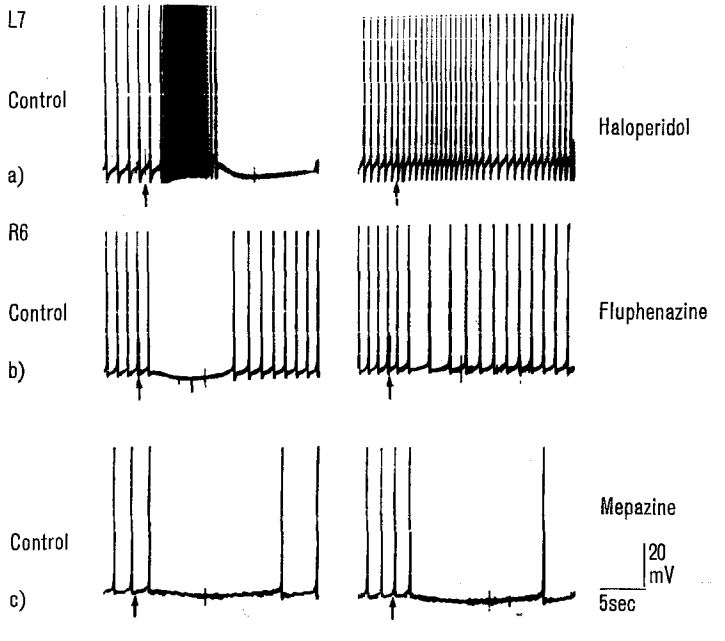

Fig. 1. Effects of drugs on the response to dopamine microelectrophoretically injected on the somatic membrane (50 nA for 1.0 sec in a and 0.5 sec in b and c, arrows). a) 50 μM haloperidol blocks D-response in cell L7. b and c) H-response in cell R6: blockade of dopamine response when fluphenazine is added to the perfusate in a concentration of 10 μM (b); the response to dopamine is not affected when the perfusate contains 10 μM mepazine (c).

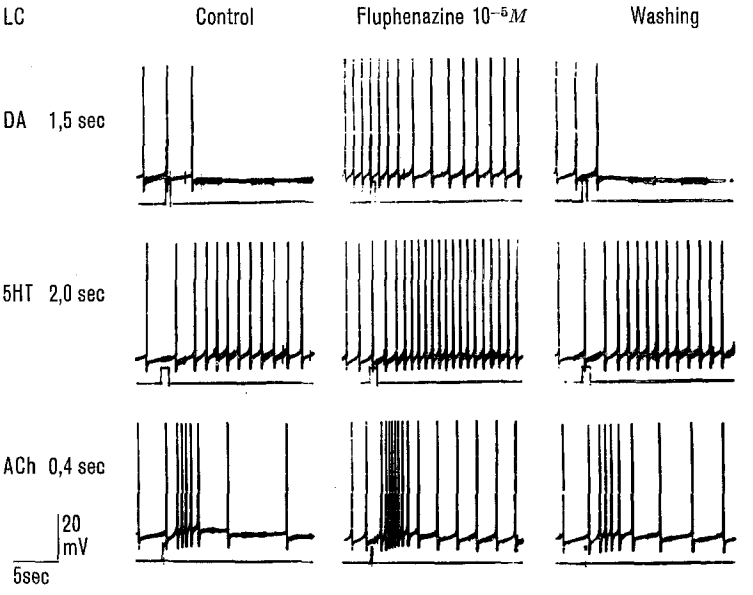

Fig. 2. Effects of 10 μM fluphenazine on responses to microelectrophoretic application of dopamine (400 nA for 1.5 sec), 5-hydroxytryptamine (250 nA for 2.0 sec) and acetylcholine (250 nA for 0.4 sec) in a spontaneously active cell type LC. The response to dopamine is blocked; this effect is reversible by washing in drug-free seawater. Iontophoretic currents from electrode to ground, measured as potential differences across a 2 MOhm resistance, are shown in lower trace of each recording.

and completely blocked within 15 min by 100 μM fluphenazine. In these neurones 10 μM mepazine had no effect (Figure 1c) and 100 μM mepazine did not impair dopamine response after 15 min perfusion. When perfusion with 100 μM mepazine was continued for further 20 min, response to dopamine started to decrease. However, this decrease did not seem to be specific for dopamine and was accompanied by impairment of the acetylcholine response which was also observed after long-lasting perfusion with 100 μM fluphenazine.

Discussion. The results provide a direct neurophysiological evidence for the blocking effect of 2 antipsychotic drugs on dopamine receptors and agree with neurochemical, pharmacological and indirect physiological observations. Poor solubility and the long latency of effect may have been responsible for an earlier finding that haloperidol was not an antagonist of dopamine on molluscan neurones[30]. D- and H-responses to dopamine involve different ionic mechanisms and are blocked selectively by tubocurarine and strychnine (D)[26,29] or ergotamine (H)[26]. Thus, it has been concluded that the effects of dopamine are mediated by 2 distinct receptors[26]. The reversible, probably competitive blocking effect of antipsychotic drugs on both types of responses in the present study might indicate that the receptors are structurally similar.

Zusammenfassung. In *Aplysia* Ganglienzellen blockierten Fluphenazine und Haloperidol in Konzentrationen zwischen 10 und 100 μM hyperpolarisierende und depolarisierende Antworten auf mikroelektrophoretisch appliziertes Dopamin. Antworten auf Acetylcholin und 5-Hydroxytryptamin blieben unbeeinflusst. Mepazine, ein Phenothiazin ohne antipsychotische Wirkung, hatte wenig Effekt. Die Ergebnisse sind ein direkter neurophysiologischer Beweis für die spezifische Blockade von Dopaminrezeptoren durch Neuroleptika.

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Effect of 6-Hydroxydopamine on Retinal Development in the Chick[1]

Attempts have been made to correlate the ontogenesis of retinal neurotransmitter systems with the development of optic function. The role of various neurotransmitter systems, particularly the cholinergic and monoaminergic systems in the retina is largely obscure, although it is well established that both cholinergic and adrenergic innervations are found in the layers and cells of the retina in most mammalian and avian species[2–6]. For example, in the developing chick retina, SHEN et al.[7] have demonstrated histochemically the presence of cholinesterase (ChE) in the prospective ganglion cells as early as the 4th day of incubation, and acetylcholinesterase was shown in the innermost layer and the amacrine cells on the 6th day. LINDEMAN[8] reported the presence of acetylcholine (Ach) and ChE activity in the retina on the 8th day. The peak rate of increment in ChE activity corresponds to the appearance of the constrictor reflex on the 19th day.

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