drug is saturable in the concentration range investigated. Similar findings on liver tissue are reported by Gigon and Guarino[8]. Own experiments (not depicted), on the time dependence of the uptake process, showed saturation after 60 min incubation time with a $t^1/_2$ of 18 min.

Figure 2a shows the dose-dependence of the negative inotropic probenecid effect. The values are given as percentage of maximal contraction force after equilibration and before probenecid was administered in the concentrations described. The maximal inhibitory effect was reached after 11.3±4.2 min showing no correlation with the time course of uptake process. After washout twice, the negative inotropic effect of probenecid was completely reversible. Moreover, it should be mentioned that no influence on the beating frequency occurred under the conditions described. The probenecid concentration used in these experiments was 2–10fold higher than serum levels in man at doses used in clinical practice. Moreover, it must be taken into account that 90% of serum probenecid is bound to plasma albumin[9], whereas the incubation medium used in our experiments did not contain any protein.

Finally, the probenecid effect on digoxin pretreated right atria was investigated. As shown in figure 2b, probenecid inhibits the positive inotropic action of digoxin ($100\ ng \cdot ml^{-1} = 0.128\ \mu M$). The values are given as percentage of maximal inotropic action after digoxin application, which was about 130% of the contraction force of untreated right atria.

The effects of probenecid on digoxin-treated as well as on untreated heart tissue in vitro may be explained as metabolic inhibition. This suggestion is supported by Pakarinen et al.[10-12], who found inhibition of $\alpha$-ketoglutarate- and succinate-oxydation in kidney mitochondria caused by probenecid. Oxygen consumption of guinea-pig kidney slices as well as of rat liver slices was also found to be decreased. A correlation of uptake and/or binding of probenecid to heart tissue and its negative inotropic action cannot be derived from the present findings, because any coincidence regarding the time course or the concentration dependence of both processes is lacking.


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## Picrotoxin-diazepam interaction in a behavioural schedule of differential reinforcement of low rates[1]


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*Summary.* In rats working in a behavioural schedule of differential reinforcement of low rates (6 or 10 sec), picrotoxin (1 mg $kg^{-1}$) decreased the number of premature responses and increased (in DRL 10 sec only) the number of rewarded responses. The effect of picrotoxin was antagonized by diazepam (2 mg $kg^{-1}$). In contrast to picrotoxin, strychnine (1.5 mg $kg^{-1}$) increased the number of premature responses.


Recent studies suggest an involvement of gamma-aminobutyric acid (GABA) processes in the control of behavioural inhibition. Interruption of GABAergic transmission by picrotoxin may either enhance inhibition elicited by aversive events[2], or despite 1 contradictory report[3], reverse benzodiazepine-induced release of responding in conflictual schedule[4,5]. In addition it has been evidenced that benzodiazepines - drugs known to release response under aversive conditions - may facilitate GABAergic transmission[6,7].

Since benzodiazepines were reported to increase response maintained under differential reinforcement of low rates (DRL) procedures[8,9], the possibility of an involvement of a GABAergic component in the control of response under such procedures was investigated. In DRL, the reinforcement schedule requires that responses must be withheld for a certain length of time in order for the response to be rewarded. The effect of picrotoxin was studied in rats working in a DRL procedure and compared to that obtained with another convulsant, strychnine, a glycine antagonist devoid of activity on GABA receptors[10]. Furthermore the capacity of diazepam to reverse picrotoxin effects was investigated.

*Material and methods.* Experiments were conducted using 3 operant boxes (placed in ventilated sound insulating cubicles) with an automatic food dispenser delivering 45 mg Noyes pellets. The boxes were equipped with 2 levers (5.5 cm above the grid floor), activation of which required a vertical force of at least 12 g to operate the microswitch.

The rats (male Wistar A.F., 150–160 g at the beginning of the study), maintained at 80–85% of their normal body weight, were first trained (16 daily sessions of 15 min) to press the right lever of the Skinner-box to obtain pellets according to a continuously reinforced schedule.

Then, DRL-6-sec, or for some rats DRL-10-sec, was introduced. These schedules contain a 6-sec (or 10-sec) period during which all bar press responses must be withheld by the rat to ensure access to the reinforcing food. Responses which occur earlier than 6 sec (or 10 sec) after the preceding bar press, reset the clock and started a new 6-sec (or 10-sec) holding period. Furthermore these DRL schedules

Effects of picrotoxin, diazepam, strychnine and picrotoxin + diazepam on the performance of rats in DRL. Results are expressed as number (mean ± SEM) of responses emitted during 15 min. Premature responses refer to presses which occur earlier than 6 sec (DRL-6-sec schedule) or 10 sec (DRL-10-sec schedule) after the preceding bar press. Reinforced responses refer to presses occurring between 6 and 10 sec or between 10 and 14 sec after the preceding bar press.

| | N | Premature responses (P) | Reinforced responses (R) | Ratio $\frac{R}{P}$ |
|---|---|---|---|---|
| DRL 6 sec | | | | |
| Controls | 7 | 75.2± 8.1 | 47.0±5.5 | 0.63±0.06 |
| Picrotoxin 1 mgkg$^{-1}$ | 6 | 52.5± 8.6 | 47.5±5.6 | 0.90±0.08** |
| Diazepam 2 mgkg$^{-1}$ | 6 | 64.5± 7.2 | 40.9±2.6 | 0.63±0.06 |
| Picrotoxin 1 mgkg$^{-1}$ + diazepam 2 mgkg$^{-1}$ | 7 | 63.3± 9.7 | 39.5±4.8 | 0.62±0.08 |
| Strychnine 1.5 mgkg$^{-1}$ | 6 | 99.1±10.7 | 43.2±2.2 | 0.43±0.04** |
| DRL 10 sec | | | | |
| Controls | 8 | 85.8± 7.5 | 36.0±3.1 | 0.42±0.05 |
| Picrotoxin 1 mgkg$^{-1}$ | 8 | 67.4± 4.1* | 48.7±2.9*** | 0.72±0.07*** |

For each parameter, statistical analyses were performed between control and treated rats using the Student's t-test: *$p<0.05$; **$p<0.02$; ***$p<0.01$.

were 4-sec limited hold. This specifies that responses occurring later than 10 sec (or 14 sec) after the last bar press were not reinforced. All rats received 35 daily DRL sessions of 15 min. Drugs were injected i.p. 30 min before the 35th session. Picrotoxin 1 mg kg$^{-1}$, strychnine 1.5 mg kg$^{-1}$, diazepam 2 mg kg$^{-1}$ were given as suspensions in a volume of 1 ml/200 g b.wt. Control rats were given distilled water.

*Results.* In the control group of rats tested in the DRL-6-sec procedure, a mean of 75.2±8.1 responses were premature and not reinforced while 47.0±5.5 presses were reinforced; a mean of 6.5±1.2 were late (non-reinforced) responses. These late responses were never found statistically modified by the drugs studied. As shown in the table, picrotoxin decreased the number of premature responses but had no effect on the rewarded responses, hence the ratio of rewarded to premature presses was significantly increased. Diazepam, ineffective by itself on DRL performance, completely suppressed picrotoxin-induced increase of the ratio between reinforced and premature responses. In contrast to picrotoxin effects, strychnine markedly increased the number of premature presses without clearly modifying the number of reinforced responses; the ratio of reinforced to premature responses was significantly decreased. In the control group of rats tested in the DRL-10-sec procedure, a mean of 85.8±7.5 responses were premature while 36.0±3.1 presses were reinforced. The late responses were negligible and not modified by drug treatment. Picrotoxin diminished the number of premature responses and augmented the number of reinforced presses, hence the ratio between the two kinds of responses were significantly increased (table).

*Discussion.* Picrotoxin reduced the number of premature and, therefore, non-reinforced responses and either did not affect or increased (in the DRL-10-sec procedure) the number of rewarded presses; this suggests that this agent enhances the ability of the animals to withhold premature responses. In agreement with previous works[2,4,5], one can assume that blockade of GABA transmission enhances some inhibitory influences upon timing behaviour, perhaps by increasing the sensibility of the rats to the aversive state that follows a non-reinforced bar press. Since DRL procedures do not seem very sensitive to motivational changes[8] and since, at this dosage, picrotoxin failed to reduce markedly either food intake[2] or, in our experimental conditions, overall response rates (less than 25%), it is unlikely that the effect of this drug on DRL response should result from a decreased food motivation.

In our study, diazepam failed to modify DRL response. This is in contrast to reports showing a disrupting action of benzodiazepines on timing behaviour by producing a greater number of premature responses[8,9]. Such discrepancies may be due to the fact that, in the other studies, holding periods were longer (10 or 15 sec) and that DRL schedules were not limited hold. However, in agreement with behavioural reports evidencing an antagonism between benzodiazepines and picrotoxin, diazepam markedly reduced picrotoxin-induced facilitation of timing behaviour. Since benzodiazepines have been reported to facilitate GABA transmission[6,7], the fact that diazepam may reverse picrotoxin facilitatory effects on DRL response may further support the involvement of a GABA component on timing behaviour.

Since strychnine produces a greater number of premature responses, blockade of glycinergic transmission may elicit disruption of timing behaviour, by releasing behaviour under inhibitory influences. Such a possibility contrasts with the inhibitory enhancing effect of strychnine in another situation[4].

In conclusion, although further pharmacological experiments are required to allow definitive conclusions, our results seem to indicate that GABAergic transmission is critically implicated in the control of behaviour during holding periods in DRL procedures.

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