lation regulates DNA synthesis in regenerating rat liver following CCl_4 administration. Little is known about whether the regulating mechanism of liver regeneration is similar following various types of liver damage. Our results suggest that the controlling role of catecholamines via the α-receptor is common in regenerating liver after CCl_4 intoxication and partial hepatectomy.

CCl_4 is a typical hepatotoxin causing centrilobular necrosis [1]. As a result of extensive studies, the initial event in the rat given CCl_4 has been established to be lipid peroxidation of the endoplasmic reticulum of the liver cell mediated by cytochrome P450 [15]. CCl_4 induces a marked necrosis during the initial 24 hr, when TS and TK levels do not increase [2]. An active hepatic proliferation involving the rise of TS and TK activities reaches its maximum at 48–72 hr after CCl_4 administration [2]. In this study, phenoxybenzamine injected at 24 hr suppressed the rise of TS and TK activities at 48 hr after CCl_4 ingestion. Therefore, the stimulation of hepatic α-receptors seems to occur around or after 24 hr following CCl_4 intoxication.

In summary, liver regeneration after administration of carbon tetrachloride was monitored by measuring thymidylate synthetase (TS) and thymidine kinase (TK) activities. The administration of an α-blocking agent, phenoxybenzamine, 24 hr after CCl_4 intoxication suppressed the rise of TS and TK, which occurred 48 hr after CCl_4 ingestion. This result suggests that liver regeneration after CCl_4 intoxication or partial hepatectomy is regulated in a similar way by catecholamines via an α-receptor.

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REFERENCES

1. Zimmerman HJ, *Hepatotoxicity, The Adverse Effects of Drugs and Other Chemicals on the Liver*. Appleton-Century-Crofts, New York, 1978.
2. Nakata R, Tsukamoto I, Miyoshi M and Kojo S, Liver regeneration after carbon tetrachloride intoxication in the rat. *Biochem Pharmacol* **34**: 586–588, 1985.
3. Tsukamoto I and Kojo S, One evidence supporting that thymidylate synthetase and thymidine kinase are the rate-determining enzymes of DNA synthesis in regenerating rat liver. *Chem Lett* 2313–2316, 1987.
4. Nakata R, Tsukamoto I, Nanme M, Makino S, Miyoshi M and Kojo S, α-Adrenergic regulation of the activity of thymidylate synthetase and thymidine kinase during liver regeneration after partial hepatectomy. *Eur J Pharmacol* **114**: 355–360, 1985.
5. Nakata R, Tsukamoto I, Miyoshi M and Kojo S, Liver regeneration in streptozotocin-diabetic rats. *Biochem Pharmacol* **35**: 865–867, 1986.
6. Tsukamoto I and Kojo S, Effect of phorbol ester on rat liver regeneration. *Biochem Pharmacol* **36**: 2871–2872, 1987.
7. Tsukamoto I and Kojo S, Effect of calcium channel blockers and trifluoperazine on rat liver regeneration. *Eur J Pharmacol* **144**: 159–162, 1987.
8. Nakata R, Tsukamoto I, Miyoshi M and Kojo S, Effect of thyroparathyroidectomy on the activities of thymidylate synthetase and thymidine kinase during liver regeneration after partial hepatectomy. *Clin Sci* **72**: 455–461, 1987.
9. Tsukamoto I, Nakata R, Miyoshi M, Taketani S and Kojo S, A new immunoblotting assay for thymidylate synthetase and its application to the regulation of enzyme activity in regenerating rat liver. *Biochim Biophys Acta* **964**: 254–259, 1988.
10. Tsukamoto I and Kojo S, Effect of glucocorticoid on liver regeneration after partial hepatectomy in the rat. *Gut* **30**: 387–390, 1989.
11. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
12. Bergmeyer HU, Neue Werte fur die molaren Extinktions-Koeffizienten von NADH und NADPH zum Gebrauch im Routine-Laboratorium. *Z Klin Chem Klin Biochem* **13**: 507–508, 1975.
13. Wallnofer H, Schmidt E and Schmidt FW, *Synopsis der Leberankheiten*. Georg Thieme Verlag, Stuttgart, 1974.
14. Thefeld W, Hoffmeister H, Busch EW, Koller PU and Vollar J, Referenzwerte fur die Bestimmungen der Transaminasen GOT und GPT sowie der alkalischen Phosphatase im Serum mit optimierten Standardmethoden. *Deutsch Med Wschr* **99**: 343–351, 1974.
15. Recknagel RO, A new direction in the study of carbon tetrachloride hepatotoxicity. *Life Sci* **33**: 401–408, 1983.

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Diffusion and absorption of (−)sulpiride and raclopride after intracerebral administration in the rat

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Intracerebral administration of drugs has often been used in animal studies to study localization of functions within the CNS, including two recent reports from this laboratory [1, 2]. The implicit assumption being that the injection is confined within the intended target in contrast to general effects produced by systemic administration. In the present report, we have investigated the diffusion within the rat striatum of two dopamine (DA) D_2 selective antagonists, (−)sulpiride [3] and raclopride [4], by estimating DA turnover at the site of injection and in adjacent brain areas.

The DA turnover should be a good indicator for this purpose as compensatory changes in DA turnover precedes functional changes, like suppression of exploratory behavior in rats (see Ref. 1). Since raclopride, in contrast to (−)sulpiride, produced an increased DA turnover also at sites distant to the injection, plasma raclopride concentrations were monitored to determine the relative role of diffusion and escape into the systemic circulation.

Materials and Methods

Animals. Adult male Sprague–Dawley rats (280–320 g) were used (ALAB Laboratorietjänst AB, Sollentuna, Sweden). The animals arrived in the laboratory at least 10 days before the experiments started, and were housed under controlled conditions of light–dark cycle (12:12 hr, lights off 06.00 a.m.), temperature (20–21°) and relative humidity (55–65%). Food (R3, Ewos, Södertälje, Sweden) and tap water was available *ad lib.*

Surgery and experimental procedures. Following general anesthesia (pentobarbital, 60 mg/kg i.p.), the animals were mounted in a stereotaxic frame and provided with guide cannulas (21G), aimed for the nucleus accumbens at the following coordinates relative bregma: AP +0.2, L +3.5 mm (neostriatum); AP +1.6, L +1.5 mm (accumbens), according to Paxinos and Watson [5]. The guides reached the dura and were fixed to the skull with acrylic dental cement. One week following surgery the hand-restrained awake animal was given a unilateral injection of (−)sulpiride (ICFI, Milan, Italy) or raclopride tartrate (Astra, Södertälje, Sweden) by means of a 31G injection needle (25G for the length of the guide and 31G for the needle penetrating into the brain parenchyma). The injections, 1 μL, were placed 4.1 and 6.8 mm below the dura for the neostriatum and the nucleus accumbens, respectively, and infused at a rate of 0.66 μL/min. (−)Sulpiride was dissolved in a minimal amount of 1 N HCl and made up to volume by adding physiological saline (pH 6.0). Raclopride was dissolved in saline only. Controls received the corresponding vehicle. The animals were decapitated 20 min following the corresponding injection, the brains were quickly removed and sliced in 2.5 mm thick coronal sections. The neostriatum and the nucleus accumbens were dissected on ice, ipsi- and contralateral to the injection, from a slice extending from approx. −0.5 to +2.0 mm relative bregma. Arterio-venous blood was collected in heparinized tubes in connection with decapitation.

Biochemistry. Dopamine and its acid metabolites dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) were measured by means of HPLC with electrochemical detection as described by Magnusson *et al.* [6]. The raclopride concentrations in plasma were determined by reversed-phase liquid chromatography after an extraction at alkaline pH. A 3 μm octadecylsilica column was used and the test compound was detected by fluorescence. The within-run precision of the method is better than 2% (10–100 ng/mL). Statistical analysis by means of appropriate parametric [7] or non-parametric methods [8], as indicated in the respective figures.

Results and Discussion

(−)Sulpiride or raclopride were administered unilaterally at one of two sites within the rat striatum: the nucleus accumbens or the dorso-lateral neostriatum. As shown in Fig. 1, (−)sulpiride (10–40 μg) produced a dose-dependent increase in DA turnover at the site of injection. There were no effects on the DA turnover contralateral to the injection, or in the alternative site of injection on the ipsilateral side. In contrast, raclopride (10–40 μg) produced an increase in the DA turnover in the whole striatum, ipsi- and contralateral to the unilateral injection (Fig. 2). Furthermore, this effect was not clearly dose-dependent, probably due to the use of maximal, or close to maximal, doses. The control levels of DA (both experiments combined) were 40.9 ± 3.2 and 66.3 ± 8.9 pmol/mg (mean ± SD) in the nucleus accumbens and in the neostriatum, respectively. There was a statistically significant decrease in neostriatal DA levels after the intra-accumbens (−)sulpiride (40 μg) injection and after the intra-neostriatal

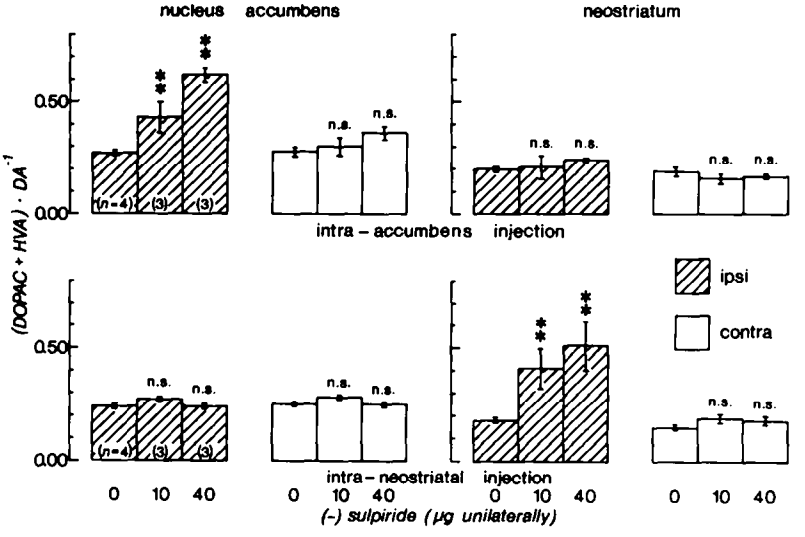

Fig. 1. Effects of unilateral intra-striatal injections of (−)sulpiride on regional striatal DA turnover. (−)Sulpiride (0–40 μg) was injected, either into the nucleus accumbens (top panel) or into the dorso-lateral neostriatum (bottom panel), as indicated in the figure, 20 min before decapitation. For further details regarding injection procedures see text. The figure shows means ± SD. Statistical analysis was performed by means of a one-way ANOVA, followed by the Dunnett's *t*-test: $F_{2,7} = 64.02$, $P < 0.01$ (ipsilateral accumbens, top panel); $F_{2,7} = 18.46$, $P < 0.01$ (ipsilateral neostriatum, bottom panel). Other F values < 4.74, $P > 0.05$. n.s. $P > 0.05$, ** $P < 0.01$.

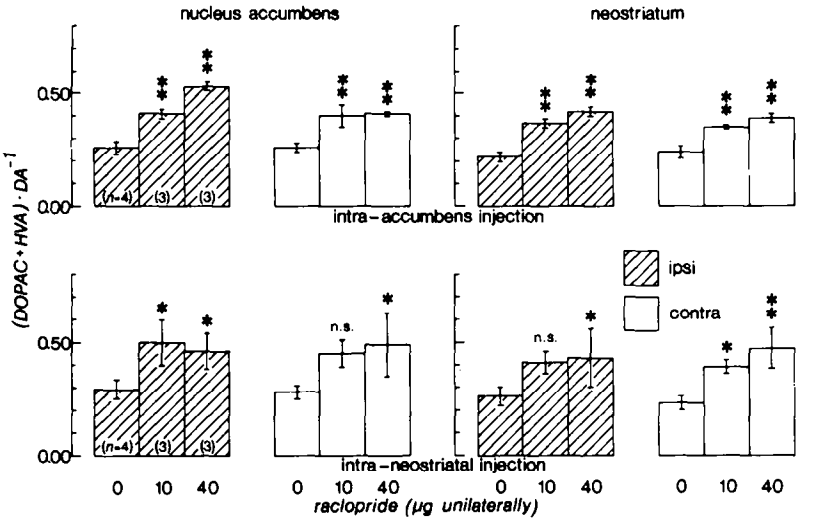

Fig. 2. Effects of unilateral intrastriatal injections of raclopride on regional striatal DA turnover. Raclopride (0–40 μg) was injected, either into the nucleus accumbens (top panel) or into the dorsolateral neostriatum (bottom panel), as indicated in the figure, 20 min before decapitation. For further details regarding injection procedures see text. The figure shows means ± SD. Statistical analysis was performed by means of a one-way ANOVA, followed by the Dunnett's t-test. All $F_{2,7}$ values > 4.74, $P < 0.05$. n.s. $P > 0.05$, *$P < 0.05$, **$P < 0.01$.

raclopride (10 μg) injection. Thus, in these two cases the quotient (DOPAC + HVA)/DA probably overestimates the DA turnover.

The bilateral injection of raclopride, 40 μg, into the nucleus accumbens resulted in a statistically significant increase in plasma raclopride levels (Fig. 3).

These results suggest that diffusion within the extracellular space is limited after an intracerebral injection and that distribution within the brain primarily is due to transport over the blood-brain barrier. At the doses used, 10–40 μg, raclopride was readily transported into blood and it should be noted that a systemic i.p. injection of 4 mg/kg of raclopride (−20 min) is needed to produce comparable plasma drug levels (unpublished observations). Calculated on body weight the intracerebral raclopride injection corresponds to approx. 0.3 mg/kg. Thus, localized intracerebral injections of highly lipophilic substances should be avoided and, if used, plasma drug concentrations should prove a sensitive indicator of distribution outside the site of injection.

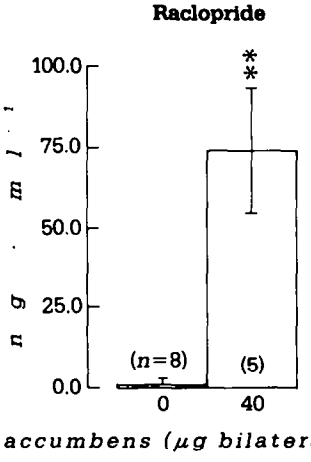

Fig. 3. Plasma concentrations of raclopride following intracerebral administration of the drug. Raclopride was administered bilaterally into the nucleus accumbens (40 μg/side), as described in the text, and the animals were decapitated 20 min later. The figure shows medians ± semi-interquartile range. Statistical comparison between concentrations in drug injected animals and the blank values of saline injected controls by means of the Mann–Whitney U-test. **$P < 0.01$.

Summary. The intracerebral injection of (−)sulpiride resulted in effects on DA turnover, confined to the site of injection with no or little evidence of diffusion to adjacent areas within the rat striatum 20 min after injection. Raclopride, on the other hand, rapidly produced effects on striatal DA turnover both locally, and at sites distal to the injection. At the same time there was a clear, and statistically significant, increase in plasma raclopride levels. Taken together these data demonstrate that diffusion within the extracellular space is limited and that drug effects, distal to a localized intracerebral injection, probably are related to the relative ease of penetration over the blood-brain barrier.

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REFERENCES

1. Ahlenius S, Hillegaart V, Fowler CJ and Magnusson O, Ipsilateral turning after local application of (−)sulpiride into the dorsal, but not the ventral, striatum of the rat. In: *Neural Mechanisms in Disorders of Movement* (Eds. Crossman AR and Sambrook MA), pp. 207–211. John Libbey, London, 1989.
2. Ahlenius S, Hillegaart V, Thorell G, Magnusson O and Fowler CJ, Suppression of exploratory locomotor activity and increase in dopamine turnover following the local application of *cis*-flupenthixol into limbic projection areas of the rat striatum. *Brain Res* **402**: 131–138, 1987.
3. Trabucci M, Longoni R, Fresia P and Spano PF, Sulpiride: a study of the effects of dopamine receptors in rat neostratium and limbic forebrain. *Life Sci* **17**: 1551–1556, 1976.
4. Köhler C, Hall H, Ögren S-O and Gawell L, Specific *in vitro* and *in vivo* binding of [^{3}H]raclopride. A potent substituted benzamide drug with high affinity for dopamine D-2 receptors in the rat brain. *Biochem Pharmacol* **34**: 2251–2259, 1985.
5. Paxinos G and Watson C, *The Rat Brain in Stereotaxic Coordinates*. Academic Press, London, 1986.
6. Magnusson O, Nilsson LB and Westerlund D, Simultaneous determination of dopamine, DOPAC and homovanillic acid. Direct injection of supernatants from brain tissue homogenates in a liquid chromatography-electrochemical detection system. *J Chromat* **221**: 237–247, 1980.
7. Winer BJ, *Statistical Principles in Experimental Design*. McGraw-Hill, New York, 1971.
8. Siegel S, *Non-parametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York, 1956.

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Muscarinic receptor antagonist activity of diacylglycerol lipase and kinase inhibitors

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Diacylglycerol (DG) is a central intermediate in lipid metabolism and is one of the two products resulting from agonist-activated phosphoinositide breakdown [1–3]. One approach used to identify the role of the diacylglycerol–protein kinase C (DG–PKC) pathway in stimulus–response coupling involves the exogenous addition of synthetic diacylglycerols or the tumor-promoting phorbol esters [4, 5]. An alternative approach has been to modify intracellular DG metabolism by the use of inhibitors. Recently, several reports [6–11] have investigated the effect of RHC 80267 [1,6-di(*O*-carbamoyl)cyclohexanone oxime)hexane], a DG lipase inhibitor, and R 59 022 (6-[2-[4-[(4-fluorophenyl) phenylmethylene]-1-piperidinyl]ethyl-7-methyl-5H-thiazolo[3,2-alpha]pyrimidin-5-one) a DG kinase inhibitor, on stimulus–response coupling. The enzyme DG lipase hydrolyzes the fatty acid ester bond at the second carbon of DG, resulting in the formation of monoacylglycerol and free fatty acid. DG kinase converts DG to phosphatidic acid by phosphorylation of the hydroxyl at the third carbon.

We reported earlier [11] that RHC 80267, R 59 022 and phorbol myristate acetate (PMA) all inhibit muscarinic-stimulated responses in guinea pig pancreatic minilobules. This, along with reports from other groups [12, 13], led to the suggestion that the activation of PKC resulting from the elevation of DG produced by these agents was responsible for the inhibition of the muscarinic receptor, presumably by a feedback mechanism. An alternative explanation involves direct inhibition by those drugs of muscarinic responses at the receptor level. Therefore, we examined the effects of RHC 80267 and R 59 022 on caerulein-stimulated cGMP formation and [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) binding to muscarinic receptors.

Materials and Methods

Guinea pig minilobule preparation. Preparation of guinea pig or rat pancreatic minilobules and conditions for cGMP studies were similar to those described earlier [11].

Membrane preparation. Rat heart ventricles and cerebral cortex membranes were homogenized in 10 vol. of 100 mM NaCl, 20 mM HEPES [4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid], 10 mM $MgCl_2$ (Buffer A) using 3 × 6 sec bursts of a Polytron homogenizer (Brinkmann Instrument, Inc., Westbury, NY) for heart and five strokes of a motor-driven Potter–Elvehjem homogenizer for cerebral cortex. Rat and guinea pig pancreatic minilobules were homogenized as described for cerebral cortex. Tissue homogenates were filtered through two layers of muslin, and centrifuged at 40,000 *g* for 20 min at 4°. Pellets were washed an additional two times, resuspended in buffer, and stored at −70° until used.

[^{3}H]*NMS Binding.* Membranes were incubated with [^{3}H]NMS buffer A (1 mL total volume) for 2 hr at 37°. Bound and free [^{3}H]NMS were separated by Millipore filtration or a Scraton cell harvester (Scraton Inc., Sterling, VA) as described previously [14]. Specific binding was defined by 1 μM atropine. Protein content, measured with bicinchoninic acid protein assay reagent (Pierce, Rockford, IL), was shown such that bound [^{3}H]NMS was less than 10% of total radioligand. Inhibition constants (K_i) were calculated from the Cheng and Prusoff equation [15]. RHC 80267 and R 59 022 were dissolved and diluted in dimethyl sulfoxide (DMSO). The final DMSO concentration in the incubation medium was 0.25%, and this had no effect on [^{3}H]NMS binding to membranes.

Chemicals. [^{3}H]NMS, 85–90 Ci/mmol, was purchased from New England Nuclear (Boston, MA). Atropine sulfate, carbamylcholine chloride, and HEPES were purchased from the Sigma Chemical Co. (St. Louis, MO). R 59 022 was bought from Janssen Life Science Products, and RHC 80267 was a gift of Mabel Hokin-Neaverson.

Results

Figure 1A shows the effects of RHC 80267 and R 59 022 on carbachol-stimulated cGMP levels in guinea pig minilobules. In the absence of inhibitor, carbachol stimulated cGMP dose dependently, with an EC_{50} of 3 μM, N = 3 (Fig. 1A). In the presence of RHC 80267 (75 μM) and