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Ca^{2+} MOBILIZATION BY VASOPRESSIN AND GLUCAGON IN PERFUSED LIVERS

EFFECT OF PRIOR INTOXICATION WITH BROMOTRICHLOROMETHANE

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Abstract—Perfused livers isolated from rats treated with $BrCCl_3$ for up to 15 min were used as an experimental tool to investigate the role of the hepatic endoplasmic reticulum in Ca^{2+} mobilization elicited by vasopressin and glucagon. $BrCCl_3$-treatment caused extensive impairment (37 to 92%) of Ca^{2+} pumps of isolated liver microsomes, while Ca^{2+} pumps of mitochondria and plasma membrane vesicles remained undamaged. In perfused livers of $BrCCl_3$-treated rats, the efflux of Ca^{2+} and the concomitant stimulation of O_2 consumption and glucose release induced by vasopressin were decreased. The extent of the decrease paralleled the duration of $BrCCl_3$-treatment. The decrease of Ca^{2+} efflux following vasopressin addition was closely correlated with the decrease of active Ca^{2+} accumulation by isolated microsomes ($r = 0.99$, $P < 0.001$). The Ca^{2+} efflux elicited by glucagon was also decreased after $BrCCl_3$-treatment, whereas stimulation of O_2 consumption and glucose release were retained. The possibility that $BrCCl_3$-treatment might impair the production of the intracellular Ca^{2+}-mobilizing messenger IP_3 is unlikely, since vasopressin still induced the formation of inositol phosphates, including IP_3, in isolated hepatocytes obtained from $BrCCl_3$-treated rats. Thus, this work supports the hypothesis that the Ca^{2+} stored in the liver ER is the major pool of intracellular Ca^{2+} available for mobilization by vasopressin, glucagon and other effectors.

The vasoactive peptides vasopressin and angiotensin II, and alpha-adrenergic agonists stimulate hepatic glycogenolysis via the activation of phosphorylase b kinase (EC 2.7.1.38) by a cAMP-independent route. These agents effect the above changes through a rise in cytosolic Ca^{2+} [1–4]. Glucagon acts by increasing cAMP, but has also been reported to elevate hepatocellular cytosolic Ca^{2+} [5, 6], which could play a role in the metabolic response to the hormone [7]. Rises of cytosolic Ca^{2+} may be achieved by an inflow of Ca^{2+} from the extracellular fluid as well as by mobilization from intracellular stores. As to the latter, uncertainty exists with respect to the subcellular localization of the hormone-sensitive pool(s) of Ca^{2+} in the liver. Earlier work emphasized the mitochondria as constituting the hormone-sensitive Ca^{2+} pool [8–11], while more recently attention has been focused on the Ca^{2+} pool associated with the endoplasmic reticulum (ER)‡ [12]. In fact, several studies with perfused liver [13–16] and isolated hepatocytes [17–19] showed that Ca^{2+}-dependent hormones (e.g. vasopressin, angiotensin II, alpha-agonists) as well as glucagon can mobilize Ca^{2+} from non-mitochondrial intracellular stores. Moreover, direct evidence of vasopressin-induced Ca^{2+} release from endoplasmic reticulum has recently been obtained by measurements of the Ca^{2+} content of endoplasmic reticulum *in vivo*, by means of electron probe microanalysis of liver tissue [20].

This communication reports on a novel approach to this problem, based on the hepatotoxicity of halomethanes. $BrCCl_3$ is actually the most toxic member of this series of compounds [21, 22].

Previous reports have shown that the *in vivo* administration of CCl_4 [23, 24] or $BrCCl_3$ [24, 25] causes an early marked impairment of the ER Ca^{2+} pumps in the liver, which precedes the functional impairment of other organelles [24–26]. On this basis, we have used livers of $BrCCl_3$-treated rats, that lack reticular Ca^{2+}-sequestering capacity, to investigate whether—and to what extent—the ER component constitutes the hormone-sensitive Ca^{2+}-pool of the liver cell.

The effects of vasopressin and glucagon on Ca^{2+} fluxes, respiration and glycolysis, were studied with perfused livers isolated from $BrCCl_3$-treated rats. As a main result, vasopressin-induced Ca^{2+} fluxes and the concomitant stimulation of respiration and glucose release were found to be impaired following $BrCCl_3$ toxicity. The impairment was correlated with the impairment of reticular Ca^{2+}-accumulating capacity.

MATERIALS AND METHODS

Animals. Male Wistar rats (190–230 g), fed on stock

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‡ Abbreviations used: ER, endoplasmic reticulum; IP, inositol 1-phosphate; IP_2, inositol 1,4-bisphosphate; IP_3, inositol trisphosphate (1,3,4- and 1,4,5-); IP_4, inositol 1,3,4,5-tetrakisphosphate; HEPES, 4-(2-hydroxymethyl)-1-piperazineethanesulphonic acid; EGTA, ethylene glycol bis(β-aminoethylether)*N,N,N',N'*-tetraacetic acid; MOPS, 3-(*N*-morpholino)propanesulphonic acid.

diet (Altromin, Lage, F.R.G.) were used. $BrCCl_3$ (undiluted) was administered by gastric intubation at the dose of 0.25 ml per kg body wt.

Haemoglobin-free rat liver perfusion. Livers were perfused as described previously, with Krebs–Henseleit buffer containing 0.2 mM Ca^{2+} (if not stated otherwise), without recirculation of the perfusate (open system) at 37° [27]. Additions were made by micropumps directly before the portal vein. Perfusate flow was about 4.0 ml/min per g wet wt. O_2 and Ca^{2+} concentration in the effluent perfusate were monitored continuously with a Clark-type electrode and a Ca^{2+}sensitive electrode (Philips IS561-Ca^{2+}), respectively [28]. The net Ca^{2+} release from perfused livers was calculated from integration of the area under the curve of the Ca^{2+}-electrode response. The latter was linear with respect to variations of Ca^{2+} concentration within the measured range, as assessed by infusing known amounts of standard Ca^{2+} solutions. Glucose concentration and lactate dehydrogenase activity in the effluent perfusate were measured as described [29].

Ca^{2+} *uptake by isolated organelles.* Liver microsomal, mitochondrial and plasma membrane fractions were prepared as described [30–32]. Ca^{2+} uptake by subcellular fractions was measured radioisotopically as content of $^{45}Ca^{2+}$ [30], or by monitoring the free Ca^{2+} concentration in the incubation system with a Ca^{2+}-selective electrode [33].

Inositol phosphate formation in isolated hepatocytes. Eighteen–20 hr before the experiment, rats were pretreated by intraperitoneal injection with 200 μCi of myo-(2-^{3}H)inositol in 0.9% NaCl [34]. Hepatocytes from both $BrCCl_3$-treated (10 min) and control rats were then prepared as reported [35]. The cells (10 mg protein/ml) were incubated in Krebs–Henseleit buffer, containing 10 mM glucose and 10 mM LiCl, and were gassed continuously with O_2/CO_2 (19/1, v/v), for 20 min before the addition of 100 nM vasopressin. After incubation for various periods of time with vasopressin, samples were withdrawn and processed according to Charest *et al.* [36] in order to measure watersoluble tritiated inositol phosphates, after separation of the individual classes (IP, IP_2, IP_3 plus IP_4) by ion exchange chromatography [37].

Other assays. Glucose 6-phosphatase activity was measured as reported [30]. Protein was determined using bovine serum albumin as standard [38].

Chemicals. Vasopressin, glucagon, ATP and glucose 6-phosphate were from Sigma (Munich, F.R.G.). $^{45}Ca^{2+}$ (165 Ci/mM) and myo-(2-^{3}H)inositol (14.3 Ci/mM) were from Du Pont-New England Nuclear (Dreieich, F.R.G.).

RESULTS

Selective impairment of Ca^{2+}*-related reticular functions by short-term* $BrCCl_3$*-treatment*

Experiments were carried out to characterize the effect of short-term $BrCCl_3$ intoxication of rats on functions of subsequently isolated liver organelles involved in the control of cellular Ca^{2+} levels. Indeed, the integrity of organelles other than the ER should be a prerequisite in order to employ the liver of $BrCCl_3$-treated rats as an experimental tool for investigating the hormonal sensitivity of the ER compartment.

Firstly, the livers of rats treated with $BrCCl_3$ (up to 20 min) did not show any appreciable cell necrosis. Like control livers, $BrCCl_3$ livers released minimal amounts of LDH (maximum values around 20 mUnits/min/g liver), during the perfusion experiments (up to 80–100 min). This indicates integrity of the plasma membrane. Furthermore, as shown in Fig. 1(a), the active Ca^{2+} accumulation by liver plasma membrane vesicles isolated from rats treated for 15 min with $BrCCl_3$ was not modified, indicating no effect on plasma membrane Ca^{2+} transport by short-term $BrCCl_3$-treatment. Similar results have been reported for CCl_4 [39].

Short-term $BrCCl_3$-treatment did not affect mitochondrial Ca^{2+}-accumulating capacity, sustained either by exogenous ATP or by endogenous substrates (succinate plus rotenone, Fig. 1b). In the presence of ATP, and in experimental conditions similar to those reported [31], mitochondria from normal and 20 min $BrCCl_3$-treated livers accumulated 69.9 ± 1.5 and 71.9 ± 2.0 nmol Ca^{2+}/mg protein, respectively, within 1 min of incubation. Further, liver mitochondria from $BrCCl_3$-treated rats exhibited an initial rate of Ca^{2+} transport and a buffering capacity toward external Ca^{2+} (down to 0.5 μM), similar to control mitochondria. Furthermore, the Ca^{2+} accumulated was kept inside the mitochondria during prolonged incubations (20–30 min), suggesting no modification of passive permeability of the mitochondrial membrane(s) to Ca^{2+} ions. These results are in agreement with previous reports [39, 40].

The inhibition of microsomal Ca^{2+} pumps following halomethane poisoning has been reported for experimental conditions (high Ca^{2+}/microsomal protein ratios, oxalate added as intravesicular Ca^{2+}-trapping agent, acidic pH of the medium) which result in a loading of microsomes with large amounts of Ca^{2+} (higher than 100 nmol/mg protein); these amounts are very likely to exceed the Ca^{2+} actually accumulated in the endoplasmic reticulum *in vivo* [41]. In order to mirror the *in vivo* conditions more closely, we have instead measured Ca^{2+} accumulation at pH 7.2, and in the presence of lower Ca^{2+} concentrations, in order to have amounts of accumulated Ca^{2+} not exceeding 10–15 nmol/mg protein. In addition, we replaced oxalate with glucose 6-phosphate, which stimulates Ca^{2+} accumulation by liver microsomes *in vitro* [30]. We suggest that glucose 6-phosphate plays a key role in Ca^{2+} accumulation by the liver cell ER *in vivo* [42–44].

As shown in Fig. 1(c), the MgATP-dependent Ca^{2+}-accumulating capacity, measured in the absence and in the presence of glucose 6-phosphate, is already significantly inhibited (30–40%) as early as 5 min after $BrCCl_3$ treatment. Slightly later, Ca^{2+}-accumulating capacity is markedly decreased (75–85% at 10 min; 90–95% at 15 min), irrespective of glucose 6-phosphate addition.

Ca^{2+} *fluxes,* O_2 *consumption and glucose release induced by vasopressin in perfused livers of* $BrCCl_3$*-treated rats*

Vasopressin causes a release of Ca^{2+} from perfused rat liver [9, 15, 16, 45]. This is followed by a Ca^{2+} reuptake, provided that enough Ca^{2+} is present in the

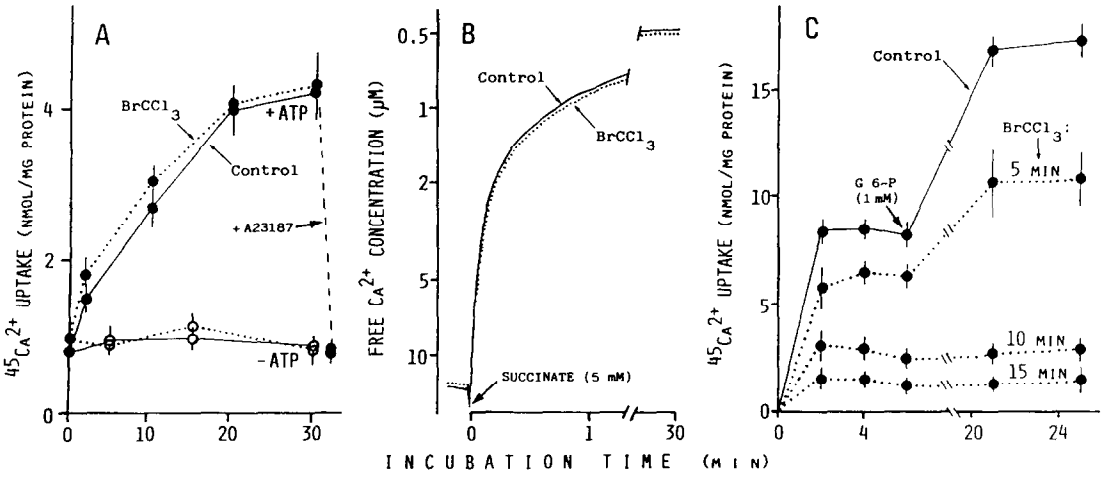

Fig. 1. Active Ca^{2+} accumulation by liver plasma membrane vesicles (A), mitochondria (B) and microsomes (C) isolated from rats treated with $BrCCl_3$. Plasma membrane vesicles and mitochondria were prepared from livers obtained from control (solid line) or 15 min-$BrCCl_3$-treated rats (dotted line) (cf. Refs 31–32). Ca^{2+} accumulation by plasma membrane vesicles was measured at 37° (cf. Ref. 30), in the presence of 10 mM MgATP, 0.2 mM EGTA and 0.18 mM $CaCl_2$ plus 21 μCi/ml $^{45}Ca^{2+}$. Membrane permeabilization to Ca^{2+} ions by the addition of the Ca^{2+} ionophore A23187 (1 μM) at 30 min of ATP-dependent Ca^{2+} accumulation resulted in immediate release of the accumulated Ca^{2+} (dashed line). Data reported are means ± SEM of three experiments. Liver mitochondria were preincubated with 5 μM rotenone; mitochondrial Ca^{2+} accumulation was then started by adding potassium succinate (5 mM, final conc.). The incubation system contained (1 ml): 1 mg protein, 10 nmoles $CaCl_2$ (added), 130 mM KCl, 10 mM HEPES (pH 7.0, 37°). Mitochondrial Ca^{2+} uptake was measured by monitoring the free Ca^{2+} concentration in the incubation system with a Ca^{2+}-electrode [33]. One experiment typical of three is shown. Liver microsomes were obtained from controls (solid line) and rats treated for 5, 10 and 15 min with $BrCCl_3$ (dotted line). The incubation system was: 100 mM KCl, 20 mM NaCl, 5 mM $MgCl_2$, 1 mM KH_2PO_4, 2 mM ATP (plus an ATP-regenerating system: 10 mM phosphocreatine and 10 μU/ml creatine phosphokinase), 20 μM $CaCl_2$ (added), 0.9 μCi/ml $^{45}CaCl_2$ and 1 mg microsomal protein/ml. Where indicated (arrow), glucose 6-phosphate (1 mM, final conc.) was added. Ca^{2+} accumulated by microsomes was measured by a rapid filtration technique [30]. In order to have the net Ca^{2+} accumulation, the amount of Ca^{2+} non-specifically bound to microsomes (i.e. in the absence of ATP), lower than 1.2 nmol Ca^{2+}/mg protein in all experiments, was subtracted. Data reported are means ± SEM of four (control) and three ($BrCCl_3$-treated, all times) experiments.

perfusion fluid, to allow Ca^{2+} entry into the liver and, in turn, a refilling of the hormone-sensitive intracellular pool(s) [10, 45, 46]. As shown in Fig. 2, in the presence of 0.2 mM Ca^{2+} in the influent perfusate, vasopressin causes a rapid Ca^{2+} release in perfused livers from control rats, followed by a Ca^{2+} re-uptake upon removal of vasopressin. This is rapidly followed by a stimulation of O_2 consumption and glucose release, as expected [15, 45, 47, 48]. The vasopressin-induced cell responses are drastically diminished in livers obtained as early as 10 min after $BrCCl_3$ treatment (Fig. 2). Table 1 shows the decrease in Ca^{2+} release by vasopressin from perfused livers taken at 5, 10 and 15 min after $BrCCl_3$ treatment, and the corresponding inhibition of the capacity of the isolated microsomes to accumulate Ca^{2+}. The two sets of data were closely correlated ($r = 0.99$, $P < 0.001$).

That the decrease of vasopressin-induced glucose release in $BrCCl_3$ livers is caused by an inhibition of phosphorylase and/or glucose 6-phosphatase activities seems unlikely. For example, glucagon still induces glucose output from $BrCCl_3$ livers comparable to control values. Glucose 6-phosphatase activity is only partially impaired by $BrCCl_3$ (−15.8 ± 2.3%, −34.9 ± 4.0% and −46.2 ± 6.1% with 5, 10 and 15 min of $BrCCl_3$-treatment, respectively; means ± SEM of three experiments).

As $BrCCl_3$ destroys the Ca^{2+}-accumulating capacity of the ER, this should result in a lesser Ca^{2+} content of this organelle. Thus, the close parallelism observed between the decrease of vasopressin-induced Ca^{2+} release and the decrease of the Ca^{2+}-accumulating capacity of ER vesicles strongly supports the idea that vasopressin indeed is mobilizing Ca^{2+} from a reticular Ca^{2+} pool.

Other factors such as an insufficient Ca^{2+}-loading of hormone-sensitive Ca^{2+} pools at the Ca^{2+} concentrations (0.2 mM) used in the perfusate and the possible impairment of the production of intracellular Ca^{2+}-mobilizing messengers (e.g. IP_3), could conceivably contribute to the reduced Ca^{2+}-mobilizing activity of vasopressin observed in $BrCCl_3$ livers. However, a similar decrease of vasopressin-induced Ca^{2+} release was also observed when the $BrCCl_3$-treated livers were perfused with a medium containing a higher Ca^{2+} concentration (1.2 mM) and the decreased Ca^{2+} release was paralleled by a concomitant decrease in O_2 consumption at 5 and 10 min after $BrCCl_3$-treatment (data not shown), and at 15 min (Fig. 3).

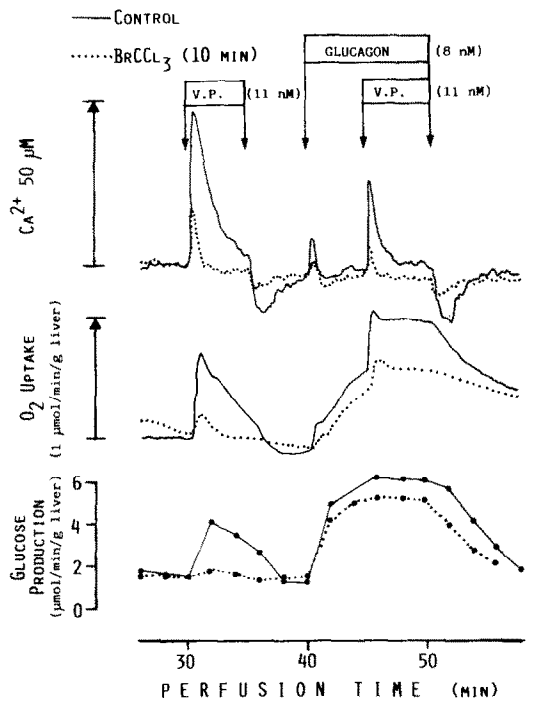

Fig. 2. Ca^{2+} fluxes, O_2 consumption and glucose release induced by vasopressin and/or glucagon in perfused livers of $BrCCl_3$-treated rats in the presence of 0.2 mM Ca^{2+}. Livers isolated from control (solid line) and 10 min-$BrCCl_3$-treated donor rats (dotted line) were perfused. Ca^{2+} in the perfusion fluid was 0.2 mM. Where indicated, 11 nM vasopressin (V.P.) and/or 8 nM glucagon were infused. Ca^{2+} (upper trace) and O_2 concentrations (middle trace) in the effluent perfusate were monitored by selective electrodes [28]. Glucose concentration in the effluent perfusate (lower trace) was measured enzymatically [29]. One experiment typical of three, both for control and $BrCCl_3$-treated livers, is shown.

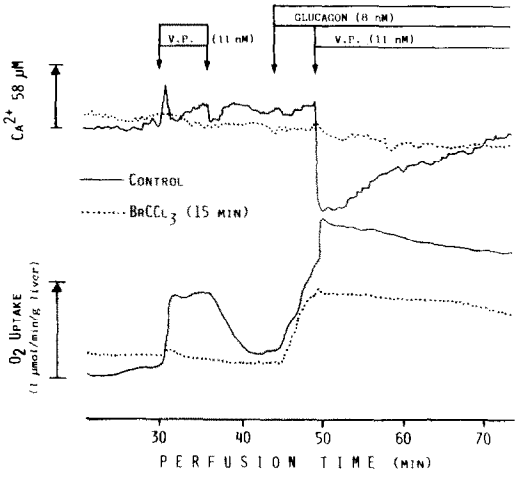

Fig. 3. Ca^{2+} fluxes and O_2 consumption induced by vasopressin and/or glucagon in perfused livers of $BrCCl_3$-treated rats in the presence of 1.2 mM Ca^{2+}. Livers from control (solid line) or 15 min-$BrCCl_3$-treated rats (dotted line) were perfused. Ca^{2+} in the perfusion medium was 1.2 mM. Where indicated, 11 nM vasopressin (V.P.) and/or 8 nM glucagon were infused. Ca^{2+} (upper traces) and O_2 (lower traces) concentrations in the effluent perfusate were monitored by selective electrodes [28]; the high medium Ca^{2+} concentration (1.2 mM) resulted in elevated noise. One experiment typical of two (control) or three ($BrCCl_3$-treated) is shown.

tocytes in the presence of lithium chloride caused an accumulation of tritiated inositol trisphosphate in the incubation medium comparable to control hepatocytes, although the pattern of accumulated phosphates (IP, IP_2, IP_3 plus IP_4) was somewhat different (Fig. 4). In particular, the accumulation of inositol polyphosphates increased linearly with time in $BrCCl_3$ hepatocytes up to 10 min after vasopressin addition. In the absence of lithium chloride appreciable amounts of inositol trisphosphates accumulated

Table 1. Impairment by *in vivo* $BrCCl_3$-treatment of vasopressin-induced Ca^{2+} release in perfused livers and of active Ca^{2+} accumulation in isolated microsomes

	Vasopressin-induced Ca^{2+} release in perfused livers		Ca^{2+} accumulation into microsomal vesicles	
	nmol Ca^{2+}/g liver	% inhibition	nmol Ca^{2+}/mg protein	% inhibition
Control	380.1 ± 19.9 (5)	0	16.9 ± 0.5 (4)	0
$BrCCl_3$ 5 min	186.3 ± 8.9 (3)	51.0	10.7 ± 0.6 (3)	36.7
$BrCCl_3$ 10 min	64.1 ± 13.9 (4)	83.1	2.7 ± 0.6 (3)	84.0
$BrCCl_3$ 15 min	18.4 ± 4.7 (4)	95.1	1.3 ± 0.3 (3)	92.3

Livers from control or $BrCCl_3$-treated rats were perfused as described in Fig. 2. The net Ca^{2+} release from perfused livers by vasopressin was calculated from integration of the area under the curve of the Ca^{2+}-electrode response corresponding to the time of vasopressin infusion (11 nM, 5 min; see Fig. 2). The Ca^{2+}-electrode response was linear with respect to variations in Ca^{2+} concentrations within the measured range, as assessed by adding known amounts of standard Ca^{2+} solutions to the effluent perfusate. Values for active Ca^{2+} accumulation by isolated liver microsomes are those reported in Fig. 1 for 25 min of incubation. Data represent means ± SEM; the number of experiments is reported in parentheses.

The possibility that the production of IP_3 is impaired following $BrCCl_3$-treatment was tested in isolated hepatocytes obtained from 10 min-$BrCCl_3$-treated rats. The addition of vasopressin to $BrCCl_3$-hepa- following the addition of vasopressin, along with a lesser formation of tritiated myoinositol (data not shown). In the absence of vasopressin, slightly higher levels of inositol polyphosphates were detected in

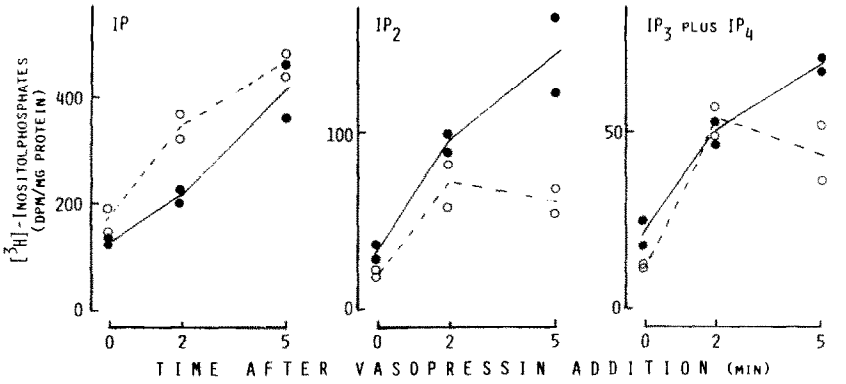

Fig. 4. Stimulation by vasopressin of the formation of water-soluble inositol phosphates in isolated hepatocytes obtained from $BrCCl_3$-treated rats. Isolated hepatocytes were obtained from controls (○) or 10 min-$BrCCl_3$-treated rats (●). Eighteen–20 hr prior to experiments 200 μCi myo-(2-^{3}H)inositol diluted in saline were injected intraperitoneally. Cells (10 mg protein/ml) were incubated in the presence of 10 mM LiCl for 20 min and then with 100 nM vasopressin. At the indicated times, samples of the incubation mixture were withdrawn and processed [37]. Two different experiments are shown, both for control and $BrCCl_3$ hepatocytes.

hepatocytes of $BrCCl_3$-treated rats. These differences in the inositol phosphate metabolism in $BrCCl_3$ hepatocytes were not further analyzed.

The marked decrease of Ca^{2+} release observed with vasopressin in perfused livers of $BrCCl_3$-treated rats was also observed with angiotensin II and ATP. The latter has been reported to effect a Ca^{2+} release from perfused rat liver, attributed to a mobilization from intracellular stores [49]. Ca^{2+} release following infusion of angiotensin II (14 nM) or ATP (50 μM) was decreased by 75 and 70%, respectively (values derived from 2 experiments), in perfused livers obtained from 10 min-$BrCCl_3$-treated rats, as compared to controls.

Thus, the selective impairment of the liver reticular Ca^{2+} pumps by $BrCCl_3$, and the associated loss of the reticular Ca^{2+} pool preclude the possibility for Ca^{2+} mobilization by vasopressin, angiotensin II and ATP. The Ca^{2+}-mobilizing capacity of these agents in normal liver can therefore be ascribed to mobilization of Ca^{2+} from a pool associated with the liver ER.

Ca^{2+} *fluxes,* O_2 *consumption and glucose release induced by glucagon in perfused livers of* $BrCCl_3$*-treated rats*

The effects of glucagon infusion were studied in perfused livers previously challenged with vasopressin, in order to compare the responses to the two hormones (Fig. 2). In control livers, the infusion of glucagon causes a small Ca^{2+} release and a marked increase in O_2 consumption and glucose release. In $BrCCl_3$-treated livers, the glucagon-induced Ca^{2+} release is markedly decreased, while O_2 consumption and glucose release are still stimulated at an extent comparable to controls. A second infusion of vasopressin in the presence of glucagon, causes a further, somewhat smaller Ca^{2+} release from control livers, accompanied by stimulation of O_2 consumption. These responses to vasopressin are diminished in $BrCCl_3$-treated livers. It has been reported [50] that the infusion of vasopressin during glucagon infusion, in the presence of 1.3 mM Ca^{2+} in the perfusate, results in a large and prolonged influx of Ca^{2+} in the perfused liver, instead of a transient Ca^{2+} release. With control livers, in our experimental conditions such a large Ca^{2+} influx under vasopressin plus glucagon infusion was absent (Fig. 2), possibly due to the relatively low Ca^{2+} in the perfusate (0.2 mM). In fact, we did observe a large Ca^{2+} influx into control livers by increasing the Ca^{2+} concentration in the perfusate up to 1.2 mM, as shown in Fig. 3; there was no Ca^{2+} influx in livers from $BrCCl_3$-treated rats (Fig. 3). This indicates a reticular involvement in the observed large Ca^{2+} influx. The Ca^{2+} influx stimulated by glucagon and vasopressin together in the perfused liver has been attributed to an enormous Ca^{2+} accumulation by a subcellular fraction containing mitochondria [50]. Others have found a considerably smaller increase of Ca^{2+} in isolated mitochondria [51]. Thus, at least two separate mechanisms may be involved in causing Ca^{2+} influx in the liver [52], one possibly related to Ca^{2+} accumulation by ER.

In the conditions employed, glucagon also mobilizes Ca^{2+} from a ($BrCCl_3$-sensitive) reticular Ca^{2+} pool (see also Ref. 16). The persistence of the stimulation of O_2 consumption by glucagon in $BrCCl_3$ livers suggests the possibility that this response is mediated through a mechanism not involving Ca^{2+} movements, as in the case of the activation of glycolysis. However, as can be seen in Fig. 5, the small Ca^{2+} release effected by glucagon appears to coincide temporally with a first, more rapid phase of stimulation of O_2 consumption, followed by a slower, prolonged stimulation. Interestingly, the progressive decrease of Ca^{2+} release observed with increasing times of $BrCCl_3$-treatment is correlated with a corresponding reduction of such a first, rapid O_2 consumption. At least the first phase of stimulation of O_2 consumption elicited by glucagon seems to be mediated through a mobilization of Ca^{2+} from a non-mitochondrial pool, likely the ER. This should result in an increase of the cytosolic Ca^{2+} levels, which might be in turn capable of raising the intramitochondrial Ca^{2+} concentration and activate Ca^{2+}-sensitive mitochondrial dehydro-

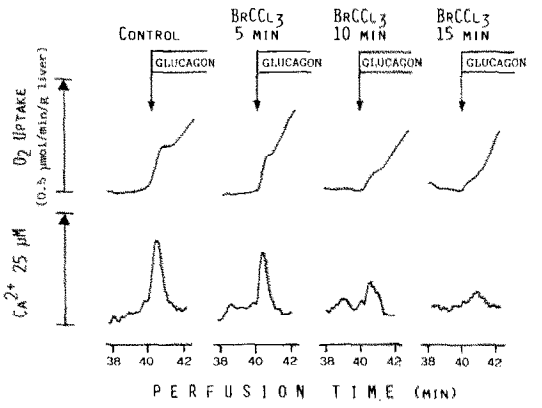

Fig. 5. Ca^{2+} efflux and rapid stimulation of O_2 consumption following glucagon infusion in perfused livers obtained from control and $BrCCl_3$-treated rats. Livers from control and 5, 10 and 15 min-$BrCCl_3$-treated donor rats were perfused. Ca^{2+} in the perfusion fluid was 0.2 mM. Where indicated, 8 nM glucagon was infused. O_2 and Ca^{2+} concentrations in the effluent perfusate were monitored by selective electrodes [28]. Typical experiments of three (control and 10 min $BrCCl_3$) or two (5 and 15 min $BrCCl_3$) are shown.

genases, as proposed by Denton and McCormack [53].

CONCLUDING REMARKS

The main feature arising from this work is that in perfused livers of $BrCCl_3$-intoxicated rats, Ca^{2+} mobilization by vasopressin, angiotensin II and ATP is drastically diminished. This closely parallels the loss of the capacity of the liver cell ER to sequester Ca^{2+} ions. Short-term $BrCCl_3$-treatment also substantially decreased Ca^{2+}-dependent O_2 uptake and glucose release by vasopressin. Ca^{2+} mobilization by glucagon was also diminished. These data support the view that vasopressin, angiotensin II, ATP and glucagon mobilize Ca^{2+} from the endoplasmic reticulum of the liver cell.

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