Inhibition by Noradrenaline and Adrenaline of the Increase in Glucose and Lactate Output and Decrease in Flow After Sympathetic Nerve Stimulation in Perfused Rat Liver: Possible Involvement of Protein Kinase C

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In perfused rat liver stimulation of the hepatic nerve plexuses increased via α_1-receptors glucose and lactate output decreased flow and caused an overflow of noradrenaline into the hepatic vein. Infusion of noradrenaline and adrenaline also elicited similar metabolic and hemodynamic alterations via α_1-receptors, whereas infusion of isoproterenol via β_2-receptors enhanced glucose output and slightly reduced lactate release without affecting flow. The influence of circulating catecholamines on the nerve stimulation–dependent changes was investigated.

Noradrenaline (100 nmol/L) or adrenaline (40 nmol/L) but not isoproterenol (1 μmol/L), which themselves caused about half-maximal alterations, strongly inhibited the nerve stimulation–induced increase in glucose and lactate output and decrease in flow but had no effect on noradrenaline overflow.

The protein kinase C activator (4β)phorbol 12-myristate, 13-acetate (100 nmol/L) but not its analog (4α)phorbol 12,13-didecanoate (100 nmol/L) strongly inhibited the metabolic and hemodynamic changes caused by nerve stimulation or noradrenaline infusion. The protein kinase C inhibitor H7 (20 μmol/L) partially prevented the inhibition of the nerve actions by noradrenaline.

The results lead us to conclude that noradrenaline and adrenaline inhibited the metabolic and hemodynamic nerve actions by means of a mechanism involving protein kinase C rather than presynaptic α-receptors or β-receptors. The catecholamines apparently increased via α_1-receptors inositol 1,4,5-trisphosphate, which in turn enhanced cytosolic Ca^{2+} and thus altered metabolism and in part hemodynamics, and diacylglycerol, which in turn activated protein kinase C and thus feedback inhibited the signal chain from α_1-receptors via G proteins to phospholipase C. (HEPATOLOGY 1992;15:477-484.)

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The adrenergic system regulates the functions of target cells either by means of innervation by sympathetic nerves or by circulating adrenaline and noradrenaline released from the adrenal glands and, in the case of noradrenaline, spilled over from terminal sympathetic synapses. The liver is innervated by sympathetic nerves, which enter the organ as a ramification around the hepatic artery and the portal vein (1-4). The liver is, of course, reached by noradrenaline and adrenaline, which could act via α_1-receptors, α_2-receptors and β_2-receptors (5, 6). The regulation of liver metabolism and hemodynamics by the sympathetic nerves has been studied *in vivo* (reviews 7, 8) and in isolated perfused rat liver *in vitro* (reviews 9, 10).

In perfused liver stimulation of the nerve bundles around the hepatic artery and portal vein increased via α_1-receptors glucose and lactate output, decreased and redistributed perfusion flow (11-13) and caused an overflow of noradrenaline into the hepatic vein (12). Infusion of noradrenaline and adrenaline also elicited via α_1-receptors, similar metabolic and hemodynamic alterations (14-17), whereas infusion of isoproterenol enhanced via β_2-receptors glucose output and slightly reduced lactate release without affecting flow (18, 19).

The nerve actions can be modulated by circulating hormones. Thus insulin antagonized the nerve stimulation–dependent increase in glucose release but had no significant effect on the shift from lactate uptake to release and on the reduction of flow, whereas glucagon did not modify the metabolic and hemodynamic nerve effects (20, 21). The influence of the α-agonists adrenaline, noradrenaline or phenylephrine and of the β-agonist isoproterenol on the nerve actions is not known. The α-agonists might cause no modifications or complex antagonistic effects either via presynaptic α_2-receptors, which can inhibit neurotransmitter release (22, 23), or via postsynaptic α_1-receptors, which by the action of diacylglycerol-activated protein kinase C can inhibit their own signal chain (24, 25). The β-agonist might have no modifying effects or cause complex amplifications via presynaptic β-receptors, which enhance neurotransmitter release (22, 23).

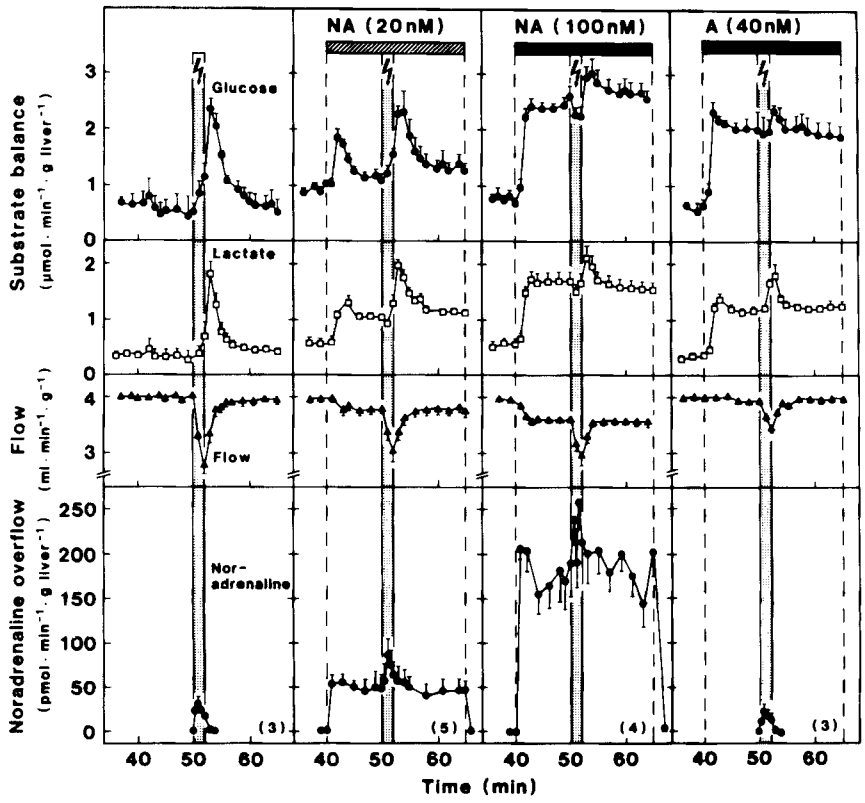

FIG. 1. Inhibition by noradrenaline and adrenaline of the alterations in substrate balance, portal flow and noradrenaline output induced by nerve stimulation in perfused rat liver. Livers were perfused through the portal vein without recirculation with Krebs-Henseleit bicarbonate buffer containing 5 mmol/L glucose, 2 mmol/L lactate and 0.2 mol/L pyruvate. Noradrenaline *(NA)* or adrenaline *(A)* were infused from the 41st to 65th min of perfusion to the final concentrations indicated. The hepatic nerves were stimulated during the 51st and 52nd min (10 Hz, 0.5 msec and 12 V) by placing the electrodes in the hilus around the portal vein and the hepatic artery. Substrate balance is given by (concentration in hepatic vein − concentration in portal vein [μmol/L × ml^{-1}] × flow (ml × min^{-1} × gm $liver^{-1}$). Values are means ± S.E.M. of the number of experiments given in parentheses.

It was the aim of this study to investigate the influence of circulating catecholamines on the nerve stimulation–dependent changes in perfused rat liver. It was found that noradrenaline and adrenaline, but not isoproterenol, inhibited the nerve stimulation–induced metabolic and hemodynamic changes without affecting noradrenaline overflow, that the protein kinase C activator (4β)phorbol 12-myristate, 13-acetate (4βPMA) (25) caused a similar inhibition and that the protein kinase C inhibitor 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7) (26) partially prevented the noradrenaline-dependent inhibition of the nerve actions. Apparently, noradrenaline and adrenaline inhibited the metabolic and hemodynamic nerve actions by means of an α_1-receptor–dependent mechanism involving protein kinase C rather than presynaptic α_2-receptors or β-receptors.

MATERIALS AND METHODS

Materials. All chemicals were of reagent grade and from commercial sources. Enzymes and BSA were purchased from Boehringer (D-6900 Heidelberg, Germany). *l*-Noradrenaline bitartrate and glucagon were from Serva (D-6900 Heidelberg, Germany); *dl*-adrenaline, isoproterenol bitartrate, 4βPMA, (4α)phorbol 12,13-didecanoate (4αPDD) and H7 were purchased from Sigma (D-8024 Deisenhofen, Germany).

Animals. Male Wistar rats (150 to 200 gm) were obtained from Winkelmann (D-4791 Borchen, Germany). At least 1 wk before the experiments, the rats were subjected to a 12 hr day-night rhythm with free access to food (standard rat diet of Ssniff, D-4770 Soest, Germany). All experiments were started between 9 and 11 AM. The animals were anesthetized by intraperitoneal injection of pentobarbital (60 mg/kg body wt).

Liver Perfusion. The liver was perfused *in situ* without recirculation through the portal vein in a 37° C cabinet using an erythrocyte-free Krebs-Henseleit bicarbonate buffer containing 5 mmol/L glucose, 2 mmol/L lactate and 0.2 mmol/L pyruvate; the medium was equilibrated with a gas mixture of 95% (vol/vol) O_2 and 5% (vol/vol) CO_2. Perfusion pressure was constant at about 10 cm H_2O with a flow rate of 3.8 to 4.2 ml × min^{-1} × gm $liver^{-1}$ under basal conditions. Flow was measured by fractionating the effluent.

Nerve Stimulation and Infusion of Signal Compounds. The hepatic nerves were stimulated with a bipolar platinum wire electrode placed around both the portal vein and the hepatic artery, which was not perfused but was still joined to the portal vein (12 V, 10 Hz, 0.5 msec). Noradrenaline, adrenaline, isoproterenol and glucagon were dissolved in saline; 4βPMA, 4αPDD and H7 were dissolved in DMSO and then diluted in the perfusion buffer containing 0.1% BSA to an appropriate

TABLE 1. Alteration in substrate balance, portal flow and noradrenaline overflow by nerve stimulation or circulating noradrenaline after previous infusion of noradrenaline, adrenaline or isoproterenol in perfused rat liver

Previous infusion	Stimulus	Alteration in glucose output (μmol · gm^{-1})	Lactate output (μmol · gm^{-1})	Portal flow (ml · gm^{-1})	Noradrenaline overflow (pmol · gm^{-1})	No. of experiments
–	Nerves	6.8 ± 1.8	3.8 ± 0.9	−3.2 ± 0.7	52 ± 5	(3/3)
Noradrenaline (100 nmol/L)	Nerves	0.5 ± 0.5[a]	0.7 ± 0.2[b]	−1.3 ± 0.4[c]	69 ± 18	(6/4)
Adrenaline (40 μmol/L)	Nerves	0.3 ± 0.2[a]	1.1 ± 0.1[a]	−1.0 ± 0.1[a]	36 ± 14	(3/3)
Isoproterenol (1 μmol/L)	Nerves	4.0 ± 0.6	2.7 ± 0.4	−2.1 ± 0.3	50 ± 8	(3/3)
–	Noradrenaline (1 μmol/L)	11.2 ± 0.6	4.5 ± 0.3	−0.9 ± 0.3		(3/–)
Noradrenaline (100 nmol/L)	Noradrenaline (1 μmol/L)	3.4 ± 0.5[a]	1.5 ± 0.1[a]	−0.9 ± 0.1		(3/–)

Data represent the areas under the curves and are taken from Figures 1 through 3. Means ± S.E.M. of the number of experiments given in parentheses (metabolism and flow/overflow). Statistics: Student's t test for unpaired values; significant differences to the controls without previous catecholamine infusion are indicated by superscripts.

[a] $p \leq 0.0025$.

[b] $p \leq 0.05$.

[c] $p \leq 0.025$.

concentration for infusion. The solutions used for perfusion had a concentration that was about 60-fold higher than the final concentration. The final concentration of DMSO was 0.01%.

Determination of Metabolites and Noradrenaline. Metabolites were measured with standard enzymatic techniques: glucose with glucose dehydrogenase (Merck glucose system) (27) and lactate with lactate dehydrogenase and ALT (28). Noradrenaline was quantitated electrochemically after separation by HPLC using the Waters system (29).

RESULTS

Rat liver was perfused without recirculation at constant pressure through the portal vein with an erythrocyte-free Krebs-Henseleit buffer containing 5 mmol/L glucose, 2 mmol/L lactate and 0.2 mmol/L pyruvate. The influence of noradrenaline, adrenaline and isoproterenol on the nerve stimulation–dependent metabolic and hemodynamic alterations was investigated.

Influence of Noradrenaline and Adrenaline on the Nerve Stimulation–dependent Alterations. Perivascular nerve stimulation (10 Hz, 0.5 msec) for 2 min increased glucose and lactate output, decreased portal flow and caused an overflow of noradrenaline into the hepatic vein (Fig. 1 and Table 1). The metabolic alterations reached a maximum (peak value) at about 3 min after the onset of stimulation; basal metabolic rates were reached again about 7 min later. The hemodynamic changes were maximal after 2 min and the noradrenaline overflow after 30 sec. All nerve stimulation–dependent effects could be inhibited by the α_1-blocker prazosin (not shown, cf. 12, 19).

Infusion of low concentrations of noradrenaline (20 nmol/L) increased glucose output transiently but enhanced lactate output and decreased portal flow constantly. The actions of noradrenaline were inhibited by the α_1-antagonist prazosin (not shown). Nerve stimulation 10 min after the onset of low level noradrenaline addition still increased glucose and lactate output, decreased portal flow and caused an overflow of neurotransmitter into the hepatic vein to the same extents as in control experiments without catecholamine infusion (Fig. 1). Infusion of medium concentrations of noradrenaline (100 nmol/L) increased glucose and lactate output and decreased portal flow constantly and about half-maximally. Now, nerve stimulation 10 min after the onset of medium level noradrenaline infusion caused clearly reduced metabolic and hemodynamic changes but still an unaltered overflow (Fig. 1, Table 1).

Infusion of low concentrations of adrenaline (40 nmol/L) increased glucose and lactate output constantly and had no effect on portal flow. The effects of adrenaline were blocked by the α_1-antagonist prazosin (not shown). Nerve stimulation 10 min after adrenaline addition elicited not only clearly reduced metabolic and hemodynamic changes but also a normal noradrenaline overflow (Fig. 1, Table 1).

Influence of Isoproterenol on the Nerve Stimulation–dependent Alterations. Infusion of low and medium concentrations of the β-agonist isoproterenol (200 nmol/L and 1 μmol/L) increased glucose output and decreased lactate output dose-dependently without affecting portal flow. Compared with control experiments the extents of the nerve stimulation–dependent increases in glucose and lactate output and the diminution of flow tended to be decreased in the presence of isoproterenol (Fig. 2 and Table 1), whereas the overflow of noradrenaline remained completely unchanged.

The submaximal increase in glucose output by 1 μmol/L isoproterenol was first lowered transiently by nerve stimulation (Fig. 2, *right panel*). This transient decrease was due to hemodynamic changes (13, 21): Nerve stimulation did not only elicit a transient reduction of total perfusion flow but also a transient intrahepatic redistribution of flow with some parenchymal areas being restricted from perfusion. Because the total amount of tissue participating in metabolism

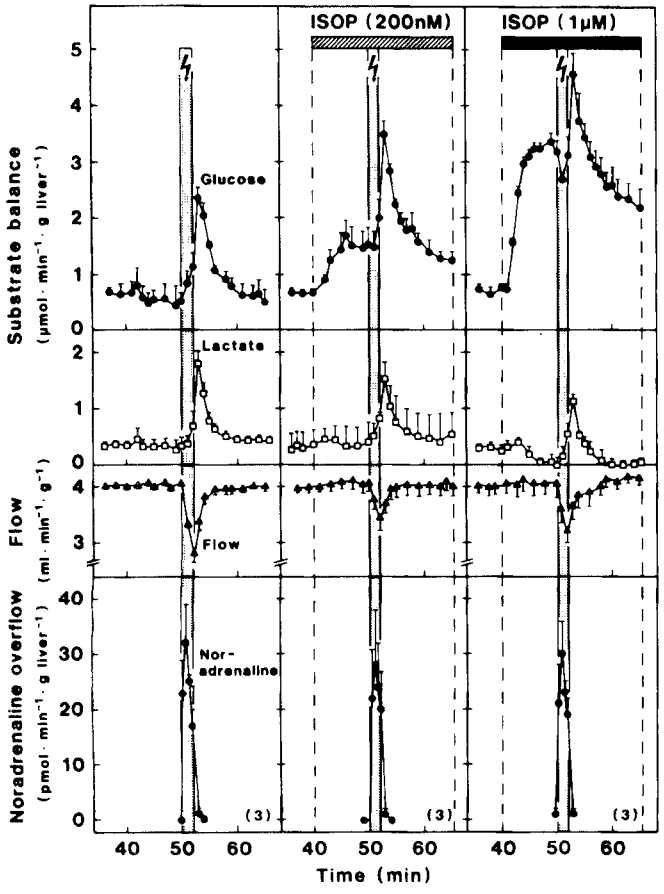

FIG. 2. Influence of isoproterenol on the alterations in substrate balance, portal flow and noradrenaline output induced by nerve stimulation in perfused rat liver. Livers were perfused and the substrate balance was determined as described in Figure 1. Isoproterenol *(ISOP)* was infused from the 41st to 65th min of perfusion to the final concentrations indicated. The nerves were stimulated during the 51st and 52nd min. Values are means ± S.E.M. of the number of experiments given in parentheses.

was reduced and because metabolism could not be increased compensatorily in the remaining perfused tissue, the isoproterenol-enhanced glucose output was reduced transiently by nerve stimulation.

Influence of Medium-level Noradrenaline on High-level Noradrenaline-dependent and Glucagon-dependent Alterations. Noradrenaline (1 μmol/L) infused for 2 min increased glucose and lactate output and decreased portal flow like nerve stimulation (Fig. 3). Medium concentrations of noradrenaline (100 nmol/L) caused similar but only half-maximal changes. The alterations in glucose and lactate output by high concentrations of noradrenaline (1 μmol/L) 10 min after the start of medium level noradrenaline infusion were strongly decreased (Fig. 3, Table 1).

Glucagon (1 nmol/L) infused for 2 min increased glucose output and decreased lactate release; portal flow remained unchanged (Fig. 3). The alterations by glucagon in glucose output were decreased by 50% and were increased in lactate balance by 100%, whereas flow was not modified 10 min after the addition of medium concentrations of noradrenaline (100 nmol/L) (Fig. 3).

Mechanism of Inhibition by Medium-level Noradrenaline and Adrenaline of Nerve Stimulation–dependent and High-level Noradrenaline-dependent Alterations. The inhibition by 100 nmol/L noradrenaline and 40 nmol/L adrenaline of the metabolic and hemodynamic alterations after nerve stimulation and 1 μmol/L noradrenaline infusion could be effected by a mechanism involving protein kinase C. Therefore the hepatic nerves (10 Hz, 0.5 msec) were stimulated, and noradrenaline (1 μmol/L) and glucagon (1 nmol/L) were infused for 2 min each in the presence of the protein kinase C–activating phorbol ester 4βPMA or of another inactive phorbol ester 4αPDD (Fig. 4). In a second series of experiments we examined whether the inhibition of the nerve stimulation–dependent metabolic and hemodynamic changes by medium concentrations of noradrenaline (100 nmol/L) could be prevented by the protein kinase C inhibitor H7 (Fig. 5).

The metabolic and hemodynamic changes by nerve stimulation and infusion of noradrenaline and glucagon (Fig. 4, *left*) were not influenced by 4αPDD (100 nmol/L) (Fig. 4 *left, broken curves*). The protein kinase C–activator 4βPMA (100 nmol/L), infused 20 min before the first stimulus, increased basal glucose and lactate output minimally and decreased portal flow slowly and slightly (Fig. 4, *right, broken curves*). The alterations by nerve stimulation and noradrenaline but not by glucagon were strongly reduced by 4βPMA (Fig. 4, *right*).

H7 (20 μmol/L) infused 10 min before noradrenaline (100 nmol/L) addition, which in turn was started 10 min before nerve stimulation, partially prevented the noradrenaline-dependent inhibition of the nerve actions. With the increases in glucose and lactate output noradrenaline no longer caused an essentially complete but only a half-maximal inhibition; yet with the decrease in flow it had only an insignificant effect (Fig. 5).

DISCUSSION

Mechanism of Action of the Inhibition by α_1-Adrenergic Agonists of the Sympathetic Nerve Effects. The general mechanisms of adrenergic regulation must also be the basis for the inhibition by α_1-agonists of sympathetic nerve actions.

Adrenergic Regulation of Metabolism and Hemodynamics. The neurotransmitter noradrenaline and the circulating hormones noradrenaline and adrenaline by binding to α_1-receptors (Figs. 1 and 3) and isoproterenol by binding to β_2-receptors (Fig. 2), as well as glucagon (Fig. 3), cause an activation of glycogenolysis and in turn of glucose output in hepatocytes. Yet the primary signal pathway of α_1-agonists is different from that of β_2-agonists and glucagon (Fig. 6). The binding of α_1-agonists at their receptors activates through a putative G protein (Gp) phospholipase C, which cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG) as second messengers (review 30, 31). In hepatocytes the increase in IP_3 causes a release of free Ca^{2+} from intracellular stores (30, 31), which activates glycogen phosphorylase kinase and in turn glycogen phosphorylase (24). The increase in DAG stimulates protein kinase C, which can provide positive and negative

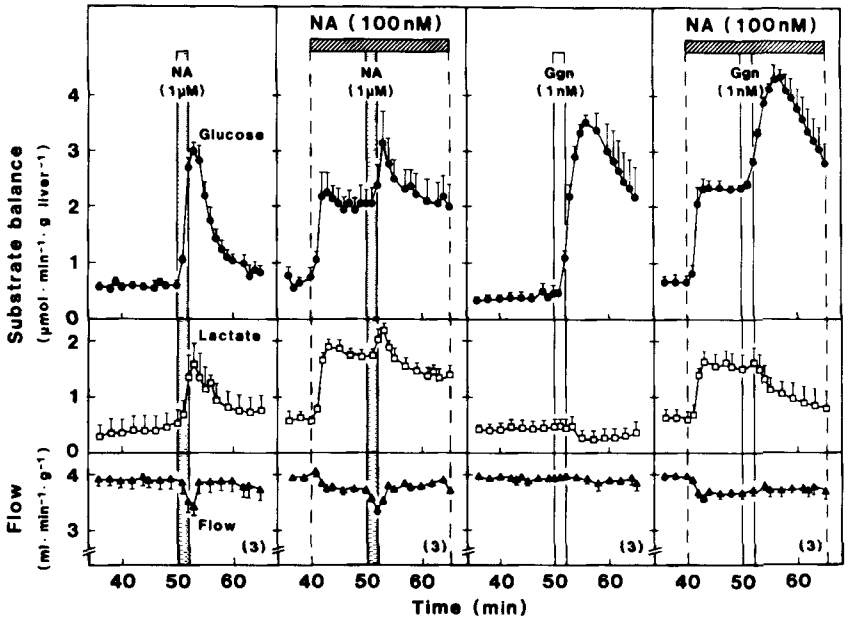

FIG. 3. Influence of medium-level noradrenaline on the alterations in substrate balance and portal flow induced by high-level noradrenaline and glucagon in perfused rat liver. Livers were perfused and the substrate balance was determined as described in Figure 1. Noradrenaline *(NA)* was infused from the 41st to 65th min of perfusion to the final concentrations indicated. Noradrenaline *(NA)* or glucagon *(Ggn)* was infused as the major stimuli during the 51st and 52nd min to the final concentrations indicated. Values are means ± S.E.M. of the number of experiments given in parentheses.

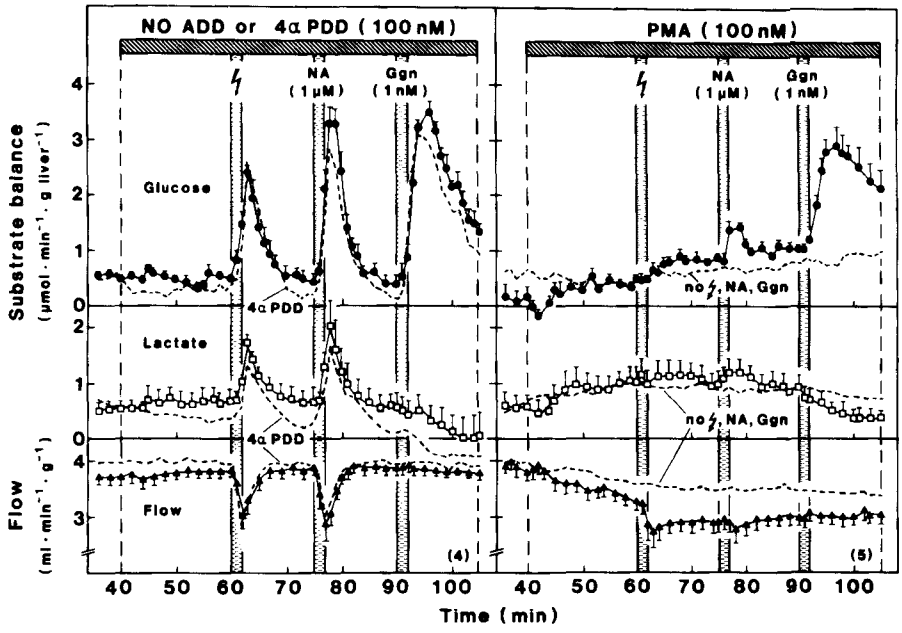

FIG. 4. Inhibition by the protein kinase C activator phorbol 12-myristate, 13-acetate of the nerve stimulation–induced and noradrenaline-induced but not glucagon-induced alterations in substrate balance and portal flow in perfused rat liver. Livers were perfused and the substrate balance was determined as described in Figure 1. (4β)Phorbol 12-myristate, 13-acetate (4βPMA) or (4α)phorbol 12,13-didecanoate *(4αPDD)* was infused from the 41st to 105th min of perfusion to the final concentrations indicated. The nerves were stimulated and noradrenaline *(NA)* and glucagon *(Ggn)* were infused to the final concentrations indicated during the 61st and 62nd, 76th and 77th and 91st and 92nd min, respectively. Values are means ± S.E.M. of the number of experiments given in parentheses.

feedback control over various steps of cell signaling systems (25, 32). In contrast, the binding of the β-agonist isoproterenol at its receptor (18, 19) and of glucagon at the putative glucagon receptor 2 (33) activates through a stimulatory G protein (Gs) adenylate cyclase, which produces cyclic AMP (cAMP) (24, 25, 33). In hepatocytes the increase in cAMP enhances glycogenolysis by an activation of the cAMP-dependent protein kinase (protein kinase A) (24) (Fig. 6).

α_1-Agonists reduce portal flow mainly by contraction

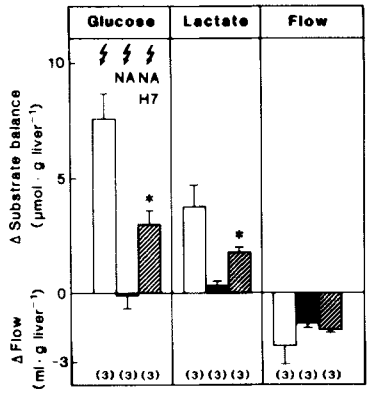

FIG. 5. Partial prevention by the protein kinase C inhibitor H7 of the inhibition by noradrenaline of the nerve stimulation–induced alterations in substrate balance and portal flow in perfused rat liver. Livers were perfused and the substrate balance was determined as described in Figure 1. H7 (20 μmol/L) was infused from the 31st to 65th min and noradrenaline (100 nmol/L) *(NA)* from the 41st to 65th min of perfusion to the final concentrations indicated. The nerves were stimulated during the 51st and 52nd min. Values are the mean alterations given as areas under the curves ± S.E.M. of the number of experiments given in parentheses. Statistics: Student's *t* test for unpaired values: *p < 0.05.

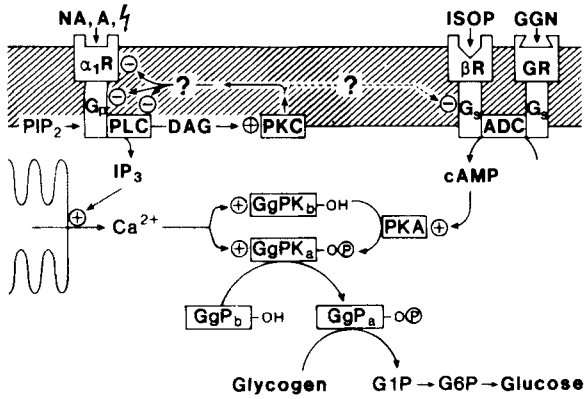

FIG. 6. Scheme of the mechanism of inhibition by noradrenaline and adrenaline of the nerve stimulation–induced alterations of metabolism and hemodynamics in liver. *A* = adrenaline; *ADC* = adenylate cyclase, *cAMP* = cyclic adenosine 3,5-monophosphate; *DAG* = diacylglycerol; *G1P* = (G6P) glucose 1(6)-phosphate; *Ggn* = glucagon, *GgP(K)* = glycogen phosphorylase (kinase); G_P and G_S = receptor-coupled G-proteins; *GR* = glucagon receptor, IP_3 = inositol 1,4,5-trisphosphate; *ISOP* = isoproterenol; *NA* = noradrenaline; PIP_2 = phosphatidylinositol 4,5-bisphosphate; *PKA* = protein kinase A; *PKC* = protein kinase C; *PLC* = phospholipase C; α_1R = α_1-receptor; *βR* = β-receptor.

of smooth muscle, which is initiated by an increase in cytosolic calcium and by a subsequent calcium-dependent/calmodulin-dependent phosphorylation of myosin light chains (34). The intracellular concentration of free calcium is regulated primarily by calcium channels and by ATP-dependent calcium pumps present in the plasmalemma and sarcoplasmic reticulum (35). α_1-Agonists, which activate receptor-dependent plasmalemmal calcium channels, also stimulate phospholipase C and thus increase the intracellular levels of IP_3 and of DAG. Both IP_3 and DAG may, in addition to plasmalemmal calcium channels, participate in mediating receptor-dependent contractions (34) because IP_3 would release Ca^{2+} from intracellular stores and DAG would activate protein kinase C, which was found to cause contraction of the rat aorta (36) and to phosphorylate myosin light chains (37).

Presynaptic Inhibitory Mechanism. The inhibition by noradrenaline and adrenaline of the nerve actions could be mediated by presynaptic receptors. Stimulation of presynaptic α_2-receptors could cause a decrease in further noradrenaline liberation from nerve endings as negative feedback control, accompanied by a limitation of calcium availability for transmitter release (22, 23). In contrast, stimulation of presynaptic β_2-receptors could cause an increase in noradrenaline liberation as positive feedback (22).

Presynaptic α-receptors or β-receptors do not appear to be involved: (a) In these experiments the overflow of noradrenaline, which can be regarded as an indication of release from nerve terminals, was not affected by α-agonists and β-agonists (Figs. 1 and 2, Table 1). (b) The prior infusion of noradrenaline (100 nmol/L) inhibited not only the alterations by nerve stimulation but also by infusion of noradrenaline (1 μmol/L), which can directly act on hepatocytes without the intervention of presynaptic receptors (Figs. 1 and 3, Table 1). (c) In a previous study with perfused rat liver (19) the α_2-receptor blocker yohimbine and the β-receptor antagonists metoprolol and butoxamine did not affect the overflow of noradrenline or the metabolic and hemodynamic alterations after nerve stimulation.

Postsynaptic Inhibitory Mechanism. The inhibition by noradrenaline and adrenaline of the nerve stimulation–dependent alterations could be mediated by postsynaptic α_1-receptors by an interruption of the signal transduction mechanism. α_1-Agonists caused a desensitization of α_1-adrenergic receptor–mediated ureagenesis in isolated rat hepatocytes (38) and rabbit vascular smooth muscle contraction (39). The desensitization was related to the activity of protein kinase C (38). Furthermore, in isolated rat hepatocytes the α_1-agonist–dependent glycogenolysis (40-42) and ureagenesis (43, 44) were attenuated by the protein kinase C–activating phorbol ester 4βPMA (25, 30-32). 4βPMA also reduced the ability of α_1-agonists to cause muscle contraction in rat and rabbit vascular smooth muscle cells (36).

In these experiments with the perfused liver the metabolic and hemodynamic changes by nerve stimulation and also by noradrenaline were almost completely blocked by the protein kinase C activator 4βPMA (Fig. 4). These results indicate that in liver the α_1-agonists inhibited the nerve stimulation–dependent alterations by means of a mechanism involving protein kinase C. In addition, the inhibition of the nerve actions by noradrenaline was partially prevented by the protein kinase C inhibitor H7 (Fig. 5). Because all known protein kinase C inhibitors including H7 are unspecific and not very effective in intact cells, the data do not prove the

involvement of protein kinase C but are further circumstantial evidence for its role. This conclusion is in line with previous findings on the attenuation of α_1-actions by protein kinase C as discussed above (25, 30-32, 36-44). This study extends previous knowledge also in that it shows that the protein kinase C–dependent feedback inhibition of the α_1-receptor–elicited signal chain, which had so far only been observed in isolated cells, is also operative in the more complex system of the intact organ.

Mechanism of Negative Feedback Inhibition of the α_1-Receptor-dependent Signal Chain by Protein Kinase C. Based on the findings that treatment with 4βPMA could inhibit α_1-agonist–dependent changes in IP_3 and cytosolic Ca^{2+} concentrations in hepatocytes (40, 44) and smooth muscle cells (45), it was suggested that 4βPMA exerted its inhibitory action on α_1-adrenergic responses through protein kinase C at the α_1-adrenergic receptor itself and also at a site close to or before IP_3 release (40, 44). One possible explanation for this mechanism might be the protein kinase C–dependent phosphorylation of α_1-receptors (45, 46), which decreases their affinity for α_1-agonists (40, 44). Another possible explanation is the protein kinase C–dependent phosphorylation of G (Gp) thought to link ligand receptor interaction to phospholipase C activation (47, 48). Moreover, a protein kinase C–elicited phosphorylation and thus inhibition of phospholipase C may be a third explanation (Fig. 6). However, the definitive mechanism of the feedback inhibition by protein kinase C remains unknown.

Function of the Inhibition by Noradrenaline and Adrenaline of the Sympathetic Nerve Actions. Half-maximally effective but not lower levels of circulating noradrenaline and adrenaline inhibited the metabolic and, to a lesser degree, the hemodynamic effects of the sympathetic hepatic nerves (Fig. 1, Table 1). This might indicate that once the circulating hormonal part of the adrenergic system is activated, no need for additional action of the nervous part exists. Probably the actions of the nervous branch are faster in onset but shorter in duration because of a developing refractoriness, and the actions of the hormonal branch are slower in onset but larger in duration taking over the actions of the nervous branch.

CONCLUSION

Noradrenaline and adrenaline inhibited the metabolic and hemodynamic nerve actions by a mechanism involving protein kinase C rather than presynaptic α-receptors or β-receptors. Via α_1-receptors the catecholamines apparently increased IP_3, which in turn enhanced cytosolic Ca^{2+} and thus altered metabolism, and the intracellular DAG level, which in turn activated protein kinase C and thus feedback inhibited the signal chain from α_1-receptors via Gp to phospholipase C (Fig. 6).

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