Transfer of Cholesterol between Phospholipid Vesicles Mediated by the Steroidogenic Acute Regulatory Protein (StAR)*

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The steroidogenic acute regulatory protein (StAR) mediates the acute stimulation of steroid synthesis by trophic hormones in steroidogenic cells. StAR interacts with the outer mitochondrial membrane and facilitates the rate-limiting transfer of cholesterol to the inner mitochondrial membrane where cytochrome P-450scc converts this cholesterol into pregnenolone. We tested the ability of N-62 StAR to transfer cholesterol from donor vesicles containing cholesterol but no cytochrome P-450scc to acceptor vesicles containing P-450scc but no cholesterol, using P-450scc activity as a reporter of the cholesterol content of synthetic phospholipid vesicles. N-62 StAR stimulated P-450scc activity in acceptor vesicles 5–10-fold following the addition of donor vesicles. Transfer of cholesterol to acceptor vesicles was rapid and sufficient to maintain a linear rate of pregnenolone synthesis for 10 min. The effect of N-62 StAR in stimulating P-450scc activity was specific for cholesterol transfer and was not due to vesicle fusion or P-450scc exchange between vesicles. Maximum stimulation of P-450scc activity in acceptor vesicles required preincubation of N-62 StAR with phospholipid vesicles prior to adding donor vesicles. The amount of N-62 StAR causing half-maximum stimulation of P-450scc activity in acceptor vesicles was 1.9 μM. Half-maximum stimulation required more than a 10-fold higher concentration of R182L N-62 StAR, a mutant associated with congenital lipoid adrenal hyperplasia. N-62 StAR-mediated transfer of cholesterol between vesicles showed low dependence on the cholesterol concentration in the donor vesicles. Thus StAR can transfer cholesterol between synthetic membranes without other protein components found in mitochondria.

The steroidogenic acute regulatory protein (StAR) mediates the rapid stimulation of steroidogenesis induced by the action of trophic hormones on the adrenal, testis, and ovary (1). The essential role of StAR in adrenal and gonadal steroidogenesis was demonstrated by its essential role in congenital lipoid adrenal hyperplasia (2, 3) and has been confirmed in knockout mice (4, 5). Early work showed that StAR is a phosphoprotein (6, 7); phosphorylation of serine 195 doubles StAR activity but is not essential for its action (8). StAR is synthesized as a short-lived 37-kDa preprotein that is cleaved to a more stable 30-kDa form upon its entry into mitochondria. However, deletion of the mitochondrial leader, which prohibits mitochondrial entry, does not diminish the activity of StAR, suggesting that StAR acts on the outer mitochondrial membrane (9–11). Biophysical studies show that StAR undergoes a pH-dependent conformational change to a partially unfolded molten globule structure (12) when it interacts with membranes (13, 14).

Although the structure of StAR has not been determined, the crystal structure of the StAR-like domain of MLN64 (15), a highly homologous protein with StAR-like activity (16, 17), and the crystal structures of the StAR homologue StAR D4 (18, 19) and phosphatidylcholine transfer proteins (20) show a β-barrel structure that appears to define a sterol-binding pocket. Direct binding assays show that StAR can bind either one (15) or two (21) molecules of cholesterol. Although this structure suggested that StAR might act as a cholesterol shuttle in the intramembranous space (15), StAR is active only on or in the outer mitochondrial membrane, and its level of activity is directly proportional to its residency time on the outer membrane (22). When the mitochondrial leader is deleted, N-62 StAR can transfer cholesterol from liposomes to the endoplasmic reticulum or to mitochondria previously treated with heat or trypsin (23). Thus the present view is that the mitochondrial leader serves to confine the activity of StAR to mitochondria and that this action requires only StAR, cholesterol, and a phospholipid membrane (24).

We previously showed that StAR preferentially interacts with synthetic phospholipid membranes that are ordered and contain cholesterol (13). We have now tested the ability of StAR to transfer cholesterol from synthetic cholesterol-rich phospholipid vesicles to cholesterol-free vesicles, using cytochrome P-450scc incorporated into the cholesterol-free vesicles as a reporter system. The ability of wild type, but not mutant StAR to foster this cholesterol transfer provides compelling evidence that only StAR, cholesterol, and a phospholipid membrane and no other proteins are required for the activity of StAR.

EXPERIMENTAL PROCEDURES

Materials—Dioleoyl phosphatidylcholine, bovine heart cardiolipin, cholesterol, glucose 6-phosphate, and NADPH were from Sigma. Dil-1

14C palmitoyl phosphatidylcholine (113 mCi/mmol) and glycerol tri[1-14C]palmitate (64 mCi/mmol) were from Amersham Biosciences and were purified by thin layer chromatography prior to use (25). The sources of other chemicals have been described previously (26).

Protein Expression and Purification—Functional human StAR lacking 62 amino acids at the amino terminus (N-62 StAR) and a corresponding inactive mutant of StAR where arginine residue at position 182 is replaced with leucine (R182L N-62 StAR) were expressed in Escherichia coli and purified as described (27). Cytochrome P-450scc...
and adrenodoxin reductase were purified from bovine adrenals (28, 29). Human adrenodoxin was expressed in E. coli and purified as previously described (30).

Measurement of StAR Activity in a Phospholipid-Vesicle Reconstituted System—This assay tests the ability of N-62 StAR to transfer cholesterol from donor phospholipid vesicles containing cholesterol but no cytochrome P-450scc to acceptor vesicles containing cytochrome P-450scc but no cholesterol. It relies on the cholesterol concentration available to the cytochrome P-450scc being below the \( K_m \) so that the rate of conversion of cholesterol to pregnenolone gives a measure of the size of the cholesterol pool transferred to the P-450scc.

Small unilamellar phospholipid vesicles were prepared from dioleoyl phosphatidylcholine and cardiolipin using a bath-type sonicator as described (31). Unless otherwise specified the cardiolipin content was 15 mol % of the total phospholipid. Cholesterol was included in some vesicles. Cytochrome P-450scc (0.01 \( \mu \)M final concentration unless otherwise specified) was incorporated into cholesterol-free vesicles by incubation for 15 min at room temperature (31). Other components of the reaction mixture (including N-62 StAR and acceptor vesicles) were then added, and preincubation was carried out for 10 min at 35 °C. Vesicles containing cholesterol were added to start the reaction under conditions where the P-450scc was catalytically active in converting cholesterol to pregnenolone. The incubation was carried out at 35 °C and comprised cholesterol-free acceptor vesicles (510 \( \mu \)M phospholipid) containing cytochrome P-450scc, cholesterol-containing donor vesicles (510 \( \mu \)M phospholipid, 20 \( \mu \)M Hepes (pH 7.4), 100 \( \mu \)M NaCl, 0.1 \( \mu \)M dithiothreitol, 0.1 mM EDTA, 2 mM glucose 6-phosphate, 2 units/ml glucose-6-phosphate dehydrogenase, 0.3 \( \mu \)M adrenodoxin reductase, 15 \( \mu \)M adrenodoxin, and 50 \( \mu \)M NADPH in a final volume of 0.25 ml. Aliquots (25 \( \mu \)l) were taken from the incubation at various times after starting the reaction with donor vesicles and added to ice-cold ethanol. The pregnenolone content of samples was determined by radioimmunoassay permitting time courses for pregnenolone synthesis to be constructed (26). For some experiments the phospholipid concentration or the timing of the addition of N-62 StAR or donor vesicles was altered, as indicated in the figure legends.

Calculation of Cholesterol Transfer by N-62 StAR—The concentration of cholesterol (\( C \)) in acceptor membranes following transfer from donor membranes was calculated from the velocity of pregnenolone synthesis using the Michaelis-Menten equation rearranged to the following form.

\[
S = \frac{V_{\text{max}}}{K_m} (V_{\text{max}} - v) \quad (\text{Eq. 1})
\]

The \( V_{\text{max}} \) and \( K_m \) values were determined separately by incorporating the P-450scc into dioleoyl phosphatidylcholine vesicles containing 15 mol % cholesterol and a range of cholesterol concentrations (25, 29). From two replicate experiments the mean \( V_{\text{max}} \) was 22.4 pmol of pregnenolone/min/pmol of P-450scc, and the mean \( K_m \) was 0.070 mol of cholesterol/mol of phospholipid.

Measurement of Phospholipid, Triglyceride, and P-450scc Transfer—To measure transfer between vesicles, neutral donor vesicles comprising dioleoyl phosphatidylcholine and 0.2 mol of cholesterol/mol of phospholipid were separated from acidic acceptor vesicles comprising dioleoyl phosphatidylcholine, 15 mol % cardiolipin, and 0.03 \( \mu \)M P-450scc by chromatography on DEAE-Sepharose CL-6B in a method comparable with that used by other workers (23, 32–34). Acceptor vesicles were labeled with 2 nCi of \( \text{d}1\)-\( \text{C} \)palmitoyl phosphatidylcholine to measure phospholipid transfer or 2 nCi of glucose 3\( \text{H} \)1\( \text{H} \)1\( \text{H} \)-palmitate (tripalmitate) to measure vesicle fusion (23). Neutral donor vesicles containing cholesterol were incubated at 35 °C for 10 min with only a linear rate of pregnenolone synthesis for 10 min with only a small proportion of the transferred cholesterol being converted to pregnenolone, as evidenced from the production of pregnenolone by P-450scc (Fig. 1). The addition of 10 \( \mu \)M N-62 StAR prior to preincubation stimulated the rate 4.9-fold over the background rate. In the presence of N-62 StAR, pregnenolone synthesis was linear from 2 to 10 min of incubation, but nonlinearity occurred after 10 min (not shown). Stimulation of cholesterol transfer to P-450scc was specific for N-62 StAR, because serum albumin and denatured N-62 StAR had no effect (Fig. 1). The addition of 10 \( \mu \)M N-62 StAR to cytochrome P-450scc in vesicles containing cholesterol had no effect on the rate of pregnenolone synthesis, demonstrating that StAR does not directly activate P-450scc (Fig. 2).

The catalytic rate constant (\( k_{\text{cat}} \)) of the cytochrome P-450scc preparation used for this study was 22.4 pmol of pregnenolone/min/pmol of P-450scc, and the \( K_m \) was 0.070 mol of cholesterol/mol of phospholipid (means from Fig. 2 and a replicate experiment). The \( k_{\text{cat}} \) is 13-fold higher than the activity shown in Fig. 1 with N-62 StAR. This demonstrates that P-450scc is working at the steady state concentration of cholesterol in the acceptor vesicles required to generate the rate of pregnenolone synthesis observed in the presence of N-62 StAR is 0.006 mol of cholesterol/mol of phospholipid. This represents 3% of the cholesterol originally in the donor vesicles. The amount of cholesterol transferred to pregnenolone during the 10-min incubation with N-62 StAR corresponds to 0.2% of the cholesterol originally in the donor vesicles. This analysis therefore suggests that when acceptor and donor vesicles are mixed in the presence of N-62 StAR, there is a rapid initial transfer of cholesterol to the P-450scc in acceptor vesicles. This maintains a linear rate of pregnenolone synthesis for 10 min with only a small proportion of the transferred cholesterol being converted into pregnenolone.

To test the function of N-62 StAR further in cholesterol transfer between vesicles, we preincubated donor and acceptor vesicles for 5 min in the presence of N-62 StAR, prior to adding NADPH to start the side chain cleavage reaction (Fig. 3). In the absence of StAR this caused a 1.7-fold increase in the background StAR-independent P-450scc activity, compared with that seen when the reaction was started by the addition of

![FIG. 1. Stimulation of cholesterol transfer between donor and acceptor vesicles by N-62 StAR.](https://example.com/figure1.png)
Tochrome P-450scc (10 nM) was incorporated into vesicles prepared from dioleoyl phosphatidylcholine and cardiolipin, and the rate of pregnenolone synthesis was determined in the absence (closed circles) or presence (open triangles) of 10 μM N-62 StAR.

Effect of cardiolipin on the stimulation of P-450scc activity by N-62 StAR

| Membrane containing cardiolipin | Background | N-62 StAR | N-62 StAR – Background |
|---------------------------------|------------|-----------|-------------------------|
| Donor + acceptor                | 0.31       | 3.2       | 2.9                     |
| Acceptor                        | 0.16       | 4.1       | 3.9                     |
| Donor                           | 3.9        | 7.1       | 3.2                     |

In the presence of N-62 StAR the rate following preincubation of donor and acceptor vesicles was only 14% higher than the rate observed when the reaction was started following the addition of donor vesicles. The StAR-mediated rate (minus background) was not altered by preincubation of donor and acceptor vesicles. This indicates that cholesterol is initially transferred rapidly to acceptor vesicles in the presence of StAR but that little or no further transfer occurs. By contrast, the background rate almost doubled with preincubation of donor and acceptor vesicles, suggesting that this rate is due, at least in part, to a continuous transfer of cholesterol from donor to acceptor vesicles.

Effect of the Cardiolipin on Cholesterol Transfer from Donor to Acceptor Vesicles—When cardiolipin was present only in acceptor vesicles, the N-62 StAR-independent background activity was decreased relative to having cardiolipin in both acceptor and donor vesicles (Table I). In contrast, the background activity was increased more than 10-fold when cardiolipin was only present in donor vesicles. N-62 StAR was able to stimulate P-450scc activity in acceptor vesicles whether cardiolipin was present in donor vesicles, acceptor vesicles or both, with activities being similar after background rates were subtracted.

N-62 StAR Does Not Stimulate P-450scc Activity by Inducing Vesicle Fusion or P-450scc Exchange between Vesicles—To confirm that N-62 StAR stimulates P-450scc activity by specifically enhancing cholesterol transfer between donor and acceptor vesicles, rates of vesicle fusion, phospholipid exchange, and P-450scc exchange were measured in the presence and absence of N-62 StAR. These measurements utilized the ability of DEAE-Sepharose to separate neutral vesicles made of dioleoyl phosphatidylcholine and cholesterol from acidic acceptor vesicles made of dioleoyl phosphatidylcholine and 15 mol % cardiolipin. N-62 StAR stimulated P-450scc activity in acceptor vesicles 4.3-fold when acceptor and donor vesicles were incubated for 10 min (Table II). Under similar conditions, vesicle fusion (measured using radiolabeled tripalmitin as a nonexchangeable marker) was small and was not stimulated by N-62 StAR. Background phosphatidylcholine transfer was higher but was also not altered by N-62 StAR. P-450scc transfer from acceptor to donor vesicles was less than the sensitivity of the assay, in both the absence and the presence of N-62 StAR, and was more than an order of magnitude too low to explain the stimulation of P-450scc activity in the presence of N-62 StAR.

Effect of StAR Concentration—The concentration dependence of the N-62 StAR-mediated transfer of cholesterol from vesicles containing cholesterol to vesicles containing cytochrome P-450scc was determined where the reaction was started by the addition of donor vesicles (Fig. 4). The data fit a hyperbolic curve, with 50% stimulation (K_1/2) occurring with 1.9 ± 0.2 μM (± S.E. from curve fit) N-62 StAR and a maximum StAR-dependent activity of 10.4 ± 0.4 times background. Thus the effect of N-62 StAR is saturable. By contrast, the R182L mutant of N-62 StAR had a minimal effect on P-450scc activity in acceptor vesicles. This mutation is a cause of congenital lipid adrenal hyperplasia and has no activity in transfected cells (3) or on isolated mitochondria (17, 22). A hyperbolic curve fitted to the data for R182L gives a K_1/2 value of 22 ± 4 μM, an order of magnitude higher than the value for wild type N-62 StAR. It was not possible to measure directly the activity of R182L N-62 StAR under saturating StAR conditions because of this high K_1/2.

Timing of the Addition of N-62 StAR to Vesicles—Adding N-62 StAR after starting the side chain cleavage reaction by the addition of donor vesicles stimulated the rate of pregnenolone synthesis 3-fold over the background rate and showed a small lag in the first minute following the addition (Fig. 5A). In contrast, adding the same concentration of N-62 StAR prior to preincubation with acceptor vesicles stimulated the rate of pregnenolone synthesis 9-fold. When N-62 StAR was preincubated with donor vesicles, and the reaction was started by adding acceptor vesicles containing P-450scc, P-450scc activity
TABLE II
Effect of N-62 StAR on tripalmitin, phospholipid, and P-450scc exchange between vesicles

Donor vesicles comprising dioleyl phosphatidylcholine and cholesterol (molar ratio to phospholipid of 0.2) were incubated with acceptor vesicles comprising dioleyl phosphatidylcholine, 15 mol % cardiolipin, and 0.05 μM P-450scc for 10 min in the absence (control) or presence of 5 μM N-62 StAR. P-450scc activity was measured as in Fig. 3 with the reaction being started by the addition of NADPH. Acceptor vesicles were labeled with tracer tri[1-14C]palmitate or di[1-14C]palmitoyl phosphatidylcholine to measure tripalmitin and phosphatidylcholine (PtdCho) transfer to donor vesicles, respectively. P-450scc transfer was below the sensitivity of the assay.

| Condition | P-450scc activity | Tripalmitin transfer | PtdCho transfer | P-450scc transfer |
|-----------|-------------------|---------------------|-----------------|------------------|
| Control   | 1.6               | 2.1                 | 11.5            | <3               |
| N-62 StAR | 7.0               | 1.7                 | 9.4             | <3               |

Fig. 4. Concentration dependence of N-62 StAR on the stimulation of P-450scc activity in acceptor vesicles. Incubations were performed as described in the legend to Fig. 1 using a range of concentrations of wild type and R182L mutant N-62 StAR. The background rate was subtracted to obtain the N-62 StAR-mediated rates of pregnenolone synthesis, and this was expressed as a ratio to the background rate (fold stimulation).

was 74% of that seen when N-62 STAR was preincubated with acceptor vesicles (Fig. 5b). Thus N-62 StAR must be preincubated with vesicles to have a maximal effect, and preincubation with acceptor vesicles fulfills this role best.

Effect of Donor and Acceptor Phospholipid Concentrations on N-62 StAR Activity—Varying the concentration of donor vesicles between 255 and 1020 μM with a constant acceptor vesicle concentration of 510 μM had little effect on N-62 StAR activity (not shown). A 9.6-fold increase in the concentration of acceptor vesicles at a constant donor vesicle concentration caused a 7-fold decrease in P-450scc activity (Fig. 6). Calculation of the amount of cholesterol transferred (see “Experimental Procedures”) revealed that it was largely independent of the acceptor vesicle concentration (Fig. 6). These results are consistent with the activity of P-450scc being determined by the concentration of cholesterol in the membrane, as has been established previously (25, 29, 31, 34). For a given amount of cholesterol transferred, P-450scc activity decreases as the ratio of cholesterol to phospholipid decreases because of an increase in the concentration of acceptor phospholipid.

Effects of Cholesterol and P-450scc on StAR Activity—The cholesterol content of the donor vesicles had a relatively small influence on the activity of N-62 StAR (Fig. 7). A 16-fold increase in the molar ratio of cholesterol to phospholipid caused a 2.3-fold increase in the background rate and only increased the N-62 StAR-stimulated rate of pregnenolone synthesis 2.1-fold.

The concentration of P-450scc in the acceptor vesicles has a marked effect on both the background rate and the N-62 StAR-stimulated rate of pregnenolone synthesis (Fig. 8). The highest ratio of StAR activity over background rate was seen with 10 nM P-450scc but did not vary greatly over the range of P-450scc concentrations tested. With 100 nM P-450scc the rate of pregnenolone synthesis in the presence of N-62 StAR was still only 26% of the maximum velocity of the P-450scc. Therefore, the activity measured at each P-450scc concentration reflects the pool of cholesterol rapidly made available to the P-450scc in the acceptor vesicles. To achieve the activity observed with 100 nM P-450scc and 10 μM N-62 StAR would require a cholesterol concentration of 0.025 mol/mol of phospholipid in the acceptor vesicles. This indicates that 12.5% of the cholesterol originally in the donor vesicles has been transferred to the acceptor vesicles.

DISCUSSION

The present study demonstrates that N-62 StAR can transfer cholesterol from donor vesicles to acceptor vesicles containing cytochrome P-450scc without the involvement of other pro-
vesicle phospholipid concentration was 510. Vesicles were added to start the side chain cleavage reaction. The donor vesicles containing 10 nM P-450scc but no cholesterol in the absence of N-62 StAR or presence of 13.6 μM N-62 StAR. The stimulation of P-450scc activity by N-62 StAR.

Our study shows that the StAR-mediated transfer of cholesterol to P-450scc is a saturable process described by a hyperbolic curve. The $K_{1/2}$ value we observed (1.9 μM) is similar to the concentration of N-62 StAR reported to stimulate pregnenolone synthesis in MA-10 cell mitochondria and mitochondria prepared from the bovine corpus luteum (10, 36). The N-62 StAR we used was expressed in E. coli (27). The purification procedure employs denaturation and refolding of the protein. This procedure appears to yield the native conformation, because the CD spectrum is indistinguishable from that obtained with N-62 StAR purified without denaturation (27), and this bacterially produced N-62 StAR is active with isolated mitochondria in vitro (17, 22, 36). Direct comparison of the activity of the bacterially expressed enzyme with the form found in tissue is not possible because active full-length STAR has never been purified from a steroidogenic tissue. The R182L mutation, which causes a severe form of congenital lipoid adrenal hyperplasia (3), increased the $K_{1/2}$ for cholesterol transfer to P-450scc by more than 10-fold without affecting the maximum amount of cholesterol transferred. This mutant is partially misfolded (27) and is inactive in transfected cells (3) and with isolated mitochondria in vitro (17, 22). However, this mutant can bind to phospholipid vesicles at low pH but with a $K_{D}$ 19-fold higher than wild type N-62 StAR (13). Thus it appears that the R182L mutant can interact with membranes and transfer cholesterol in an artificial system but with much lower efficiency than wild type STAR. Similarly, in measurements of the interaction of N-62 StAR with a fluorescent cholesterol derivative, the inactive A218V mutant displayed 7.2-fold weaker binding (21). Thus biological activity does not correlate directly with binding in vitro.

Small unilamellar vesicles similar to those used in the present study have a slow rate of cholesterol exchange with a half-time of about 1 h, which is not increased by the presence of P-450scc. The results of our study and those of others (34) show that there is little vesicle fusion over the typical time period used in the current study (34). P-450scc spontaneously incorporates into these vesicles with its adrenodoxin-binding site facing the exterior (28, 31, 37). Rapid exchange of P-450scc between vesicles has been reported at high P-450scc concentrations (0.25–0.5 μM) but is markedly reduced by the presence of acidic phospholipids in the vesicle membrane (34, 38). Our results showing a low StAR-independent background P-450scc
activity when cardiolipin is present in the acceptor vesicle membrane support this, with P-450scc exchange between vesicles containing a low P-450scc concentration being too small to measure. It is likely that the background, StAR-independent cholesterol transfer we observed is due, at least in part, to slow cholesterol exchange, because the amount of cholesterol transferred to acceptor vesicles depends on the time they are incubated with donor vesicles. The relevance of this to the StAR-independent steroidogenesis seen in patients with congenital lipoid adrenal hyperplasia (3) is not clear.

To have full activity in terms of the amount of cholesterol transferred to P-450scc, it appears that N-62 StAR must be preincubated with a vesicle membrane. The acceptor membrane containing P-450scc seems to fulfill this role better than the donor vesicle membrane. This might relate to changes in vesicle properties, such as the asymmetry created by the presence of P-450scc. It is also possible that the membrane induces a conformational change in StAR that is a prerequisite for cholesterol binding. The crystal structures of MLN64 (15), StAR D4 (18, 19) and phosphatidylcholine transfer protein (20) show that there is not enough room for cholesterol to enter the sterol-binding pocket so that a conformational change is necessary for cholesterol binding. Similarly, spectroscopic data indicate that StAR undergoes a pH-dependent conformational change (12–14) that may be related to the conformational change needed for cholesterol binding.

The amount of cholesterol transferred from donor to acceptor vesicles by N-62 StAR is relatively independent of the concentration of the donor and acceptor vesicles and the presence or absence of cardiolipin in the vesicle membranes. The cholesterol content of the donor vesicle also does not greatly influence N-62 StAR-mediated cholesterol transfer, particularly at high cholesterol concentrations; a 16-fold decrease in cholesterol concentration caused a 2.1-fold decrease in the amount of cholesterol transferred by N-62 StAR. This observation suggests that the cholesterol content of the outer mitochondrial membrane has little influence on cholesterol transfer to the inner membrane and is consistent with the previous findings that it is the concentration of StAR and its residency time in the outer mitochondrial membrane that primarily determine the rate of cholesterol transfer to the inner mitochondrial membrane (22).

Increasing the P-450scc concentration in acceptor vesicles increases the amount of cholesterol transferred to acceptor vesicles by N-62 StAR. Because StAR works without penetration of the inner mitochondrial membrane (22), a direct interaction of StAR and P-450scc seems unlikely. It is likely that P-450scc induces structural changes in the acceptor vesicle membrane that facilitate StAR action, as mentioned above.

Our study shows that following a rapid transfer of cholesterol when the vesicles are mixed, there is little subsequent N-62 StAR-mediated cholesterol transfer even though a gradient of cholesterol remains between donor and acceptor vesicles and the concentration of N-62 StAR is saturating. The highest stoichiometry for molecules of cholesterol transported per molecule of N-62 StAR in this study was 2.8, observed with a low concentration of StAR (0.7 μM; Fig. 4). With a higher P-450scc concentration and a nearly saturating level of N-62 StAR (Fig. 6), the stoichiometry of transfer was 1.8 molecules of cholesterol/molecule of N-62 StAR. By contrast, experiments in mouse adrenal Y-1 cells suggest that each molecule of StAR may facilitate the transfer of up to 400 molecules of cholesterol (39). The ability of StAR to transfer multiple molecules may require factors not present in the reconstituted system we are using.

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